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PURPOSES

The main purpose of the Physiological Reviews is to furnish a means whereby those interested in the physiological sciences may keep in touch with contemporary research. The literature, as every worker knows, is so extensive and scattered that even the specialist may fail to maintain contact with the advance along different lines of his subject. The obvious method of meeting such a situation is to provide articles from time to time in which the more recent literature is compared and summarized. The abstract journals render valuable assistance by condensing and classifying the literature of individual papers, but their function does not extend to a comparative analysis of results and methods. Publications such as the Ergebnisse der Physiologie, the Harvey Lectures, etc., that attempt this latter task, have been so helpful as to encourage the belief that a further enlargement of such agencies will be welcomed by all workers. It is proposed, therefore, to establish a journal in which there will be published a series of short but comprehensive articles dealing with the recent literature in Physiology, using this term in a broad sense to include Bio-chemistry, Bio-physics, Experimental Pharmacology and Experimental Pathology.

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THE AMERICAN
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STUDIES ON THE CEREBROSPINAL FLUID

VII. A Study of the Volume Changes of the Cerebrospinal Fluid after Adrenalin, Pituitrin, Pilocarpine and Atropine

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Received for publication February 7, 1921

In an earlier paper the methods employed in the study of the movements of the cerebrospinal fluid were carefully reviewed and discussed at considerable length (1). It was pointed out that the *outflow method* so commonly employed was unsatisfactory for the following reasons: *1*, The preliminary drainage of the fluid reduced the pressure within the skull, so that new formation might or might not be by the normal mechanism. *2*, Since contained in so complicated a system it obviously is impossible to remove all the fluid present at the beginning of the experiment and thus to differentiate between preformed and newly formed fluid. A part of the fluid flowing from the needle may thus be fluid flowing from a higher to a lower level under the influence of gravity, or of other pressure. *3*, It was impossible by this method to judge the effect of arterial and venous change and the readjustment, for the fluid which had fallen from the tube was lost, and could not reënter the canal if the opportunity presented itself. *4*, It is possible for blood to accumulate in the sinuses, displacing fluid through the needle, thus setting up a pseudo-secretion without any new formation of fluid. *5*, It is difficult, if not impossible, to control the effects of respiratory movements.

The *manometric method* was considered more suitable but open to some criticism: *1*, Pressure conditions are such as to favor the absorption of fluid along the natural channels. Thus the rise in pressure

might mean new formation, while the fall which followed might mean absorption along the normal channels by the usual mechanism. It was shown, however, that the fall occurred just the same with the jugulars ligated as with the jugulars open, and therefore, since the jugular are conceded to be the pathway of absorption, absorption could not have been the cause of the fall. *2*, It must be conceded that some of the fluid of the body can be formed against high, others only against low pressure. It is possible, therefore, that the pressure of formation of the fluid might be so low as to be checked by the pressure found at times in the manometric method of determination. It was with the idea of eliminating this possible criticism that the present work was undertaken.

The method of study employed is open to some of the errors pointed out for the outflow method. It reduces the pressure, it is slow in revealing pressure changes both when pressures are rising and falling, but especially in the latter case it does not differentiate between preformed and newly formed fluid, but *it does show that under the influence of drugs, if the fluid pressure is kept a constant, outflow and inflow are equally a part of the picture* of the action, and thus under the influence of drugs there is absolutely no evidence of new formation of fluid. This then is further proof of our contention that the increased outflow and increased fluid pressure observed are to be explained upon a purely mechanical basis, the result of changes in the arterial or venous pressures or both.

Method. The animal was anesthetized by the intraperitoneal injection of chloretone in oil in dosage sufficient to produce complete anesthesia and immobilization in from 15 to 30 minutes. The animal was then tracheotomized and no further anesthetic was required. Complete immobilization is absolutely necessary in this method because even the slightest voluntary movement is liable to puncture the medulla or cause hemorrhage into the canal, rendering the animal unfit for further study.

The amount of chloretone needed is not sufficient materially to reduce the reactivity of the centers, for the graphs all show typical reactions to the drugs which were subsequently given. Arterial and venous pressures were taken as described in the earlier paper. In most cases we recorded the venous change by direct readings, as well as by a tambour adjusted to the drum. The tambour record is only approximately, the number readings of venous pressure absolutely correct. In the case of the fluid we recorded, instead of pressure, the volume

changes. A short, wide hypodermic needle was attached by means of a short piece of gum tubing to the bottom aperture of a Mariotte bottle having a transverse diameter of 50 mm. and a total capacity of 150 cc. In a bottle of this size the addition of 1 cc. of fluid raises the level by almost exactly 0.5 mm. Thus since the amount of fluid displaced amounts rarely to more than 2 cc., the change in level between the lowest and the highest point will be only about 1 mm., an amount insufficient to produce any appreciable difference in pressure. After the needle was thrust through the atlanto-occipital membrane the bottle was placed on a stand so adjusted that the bottom was level with the midline of the canal, and so inclined that the side to which the tubular aperture was fused was 4 mm. lower than the other. This inclination was established in order to insure that fluid and not air would reënter the canal as long as any was present in the bottle. In this arrangement when the ligament is punctured fluid flows from the canal into the bottom of the bottle until an equilibrium is established, ordinarily about 2 cc. enter. Ten minutes were allowed for the establishment of the equilibrium, then the upper aperture of the bottle was connected by means of air tight tubing to a large-bowled but very delicate tambour covered with moderately heavy rubber dam stretched barely taut across the bowl. Since the bowl was large (5 cm.), the rubber almost slack and the volume change small, the amount of tension exerted by the rubber stretched by the admission of fluid into the bottle is negligible. The writing point of the tambour wrote on the drum above the blood pressure and parallel with the venous pressure.

By this means we were able to measure accurately graphically the volume increase of fluid within the bottle without modifying the pressure, thus permitting the fluid to flow from or into the canal with the minimum of interference. In an experiment in which venous pressure increased and the fluid outflow increased there was room in the bottle for the increase without altering the pressure; when the venous pressure returned to the normal the fluid was available to reënter the canal if the opportunity was offered. In this way we were able to measure *the volume change in the fluid in the canal* plus a reservoir with a total capacity of about 200 cc.; the bottle, rubber tubing and tambour bowl. We standardized the system after each experiment by clamping the tube between the Mariotte bottle and the needle, and injecting known amounts of water into the bottle, and recorded the amount of change in the tambour lever in the usual manner. If there is new formation under the influence of drugs the fluid should flow out into the bottle in amount

equal to the amount formed, then when vascular readjustment takes place the fluid should remain in the bottle for there has been no increase in pressure in the system to force it back into the canal as was the case where the manometer was used. As we see the conditions here *if the fluid failed to return to the canal*, it would not necessarily constitute proof that there had been new formation, because there might be lacking within the canal the force to compel the return of the fluid, but *if the fluid returns, then this method gives the required proof that there has been no new fluid formation*, otherwise there should be no room in the canal for the fluid forced out. As stated before, this method is open to the criticism that the pressure within the canal is reduced.

We did not feel that many experiments need be performed, especially after we found that the results agreed absolutely with those already published secured by the other method. The only difference in results by this method from those already secured by the other method is the constant one obviously to be expected; viz., venous rise and fluid rise will be more or less synchronous because we are dealing with a definite positive force—the rise in venous pressure—in some cases the rise is slow because of the resistance encountered by the fluid in the needle and at other points in the system. The greatest delay, however, comes in the fall in the fluid volume, because it is to be expected that equalization of differences in pressure under the conditions would be slow, because there could be no great negative pressure developed within to suck the fluid back, nor is there any positive force on the outside to drive it back as in the manometer method, where a great difference in the level of fluid on the two sides of the manometer is developed. Thus the readjustment is slow and tedious in some cases—but in others surprisingly rapid.

Adrenalin. The position taken in the earlier paper was that, in agreement with some earlier writers, we were of the opinion that the changes reported in fluid pressure by the manometric, and the increased outflow by the drop method after the injection of adrenalin are due entirely to vascular changes and readjustments within the skull. The results are exactly the same whether the vagi be cut or paralyzed with atropine. With the rise in arterial pressure in the dog injected with adrenalin comes a very pronounced rise in venous pressure. If the fluid is flowing out of the needle inserted into the fourth ventricle there is a marked temporary increased outflow, most marked while venous pressure is rising, but which may persist so long as arterial and venous pressures are higher than normal. This outflow does not

necessarily cease when the pressures cease to rise. This outflow is seen even if the sinus pressure is kept as nearly constant as possible by permitting the sinus to bleed during the action of the drug. If the dural canal is connected with a water manometer the pressure in the fluid manometer closely follows the venous pressure, the only difference being that the fluid pressure continues to rise after arterial and venous pressures have begun to fall and never reaches the same height. These differences have been adequately explained on mechanical grounds.

To support our earlier claims that in the case of adrenalin we are dealing with mechanical changes we present figure 1. This is a single tracing typical of several others made by this method. In others the return to normal was better than in the case presented. As can be seen from the graph, the arterial curve is typical of adrenalin administered to atropinized dogs. The rise is steep, and practically complete in 15 seconds. A slight rise continued for the next 11 seconds but this is due largely to increased strength of the heart resulting in increased oscillation of the mercury column. Thus the true vasomotor effect is complete in 15 seconds. The venous rise is complete in 16 seconds, and begins to fall away immediately. This fall continued slowly but steadily for several minutes. There is no doubt that there was a slight leak in the venous tambour system, but the venous pressure readings on the second line from the bottom are correct. The fluid volume, as can be seen from the graph, increased for 90 seconds, and when the maximum was reached between 2 and 2.5 cc. had entered the bottle from the canal, an amount which raised the level of the fluid in the bottle by not more that 1.25 mm. Following this with the arterial and venous readjustment, this fluid with the exception of about 0.6 cc. returned to the canal. It must be noted that arterial pressure was 10 mm., the venous pressure 38 mm. higher at the end than at the beginning of the observation, a difference sufficient to explain why the 0.6 cc. failed to return to the canal, its place being occupied by the increased amount of blood in the sinuses under the increased pressure. In two other experiments, graphically less satisfactory, the return was perfect in both cases.

Since the conditions in this experiment reproduce the conditions during the period of active outflow in the typical outflow experiment and since it is perfectly clear that the fluid returns to the canal, we conclude that the fluid which escapes from the canal by the outflow method during the pressor phase of adrenalin activity is not newly formed but is preformed fluid mechanically forced out of the canal by increased arterial and venous pressure. Where conditions are absolutely

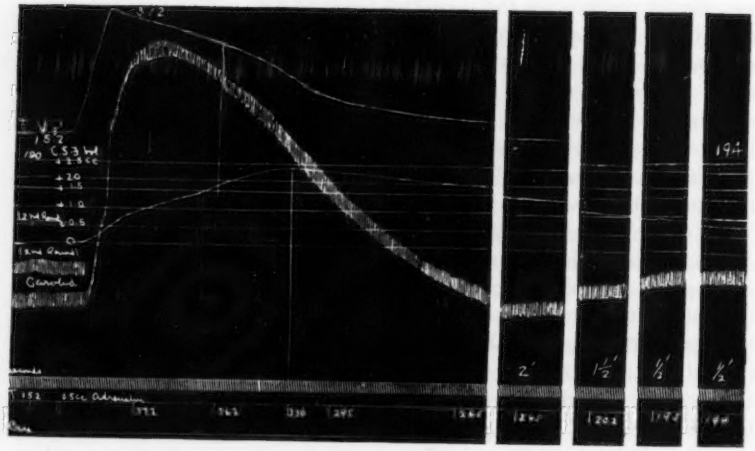

Fig. 1. The effect of adrenalin on venous pressure and fluid volume

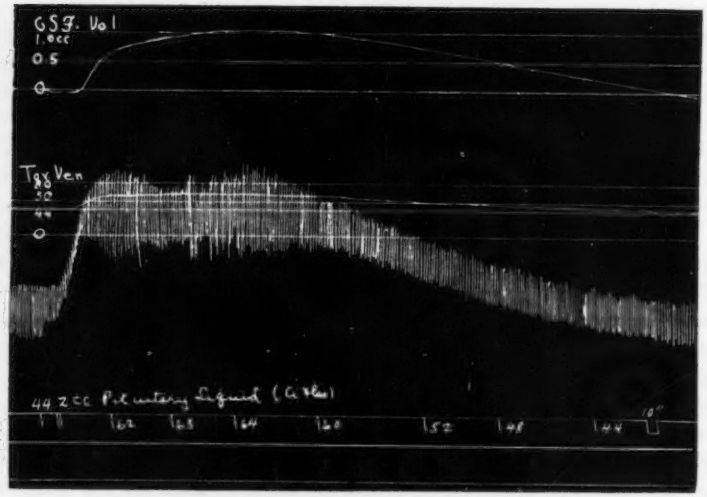

Fig. 2. The effect of the pituitary liquid on venous pressure and fluid volume

satisfactory, outflow and return are equal, and since by this method the pressure is kept under the normal so that the question of too high a pressure for formation cannot enter, as was the case by the manometer method, we conclude that we have conclusive evidence *that there is no new formation* of fluid *during* any phase of *adrenalin action.* The increased outflow, and the increased pressure of the fluid being due only to the alteration of the amount of blood and the changes of the pressure in the arteries and veins within the skull.

Pituitary extract. When the same method of study was applied to extract of the pituitary, equally striking results were obtained. Of four experiments the most satisfactory is shown in figure 2.

In this case the arterial pressure curve is a typical curve for a normal dog; a steep rise with a certain degree of slowing, which persisted after the arterial rise had disappeared, and a slow return of the blood pressure almost to normal after about 20 minutes. The venous pressure rose at first abruptly from 44 to 62, then more slowly to 64, and then fell gradually to 44. The fluid curve was almost exactly synchronous, a very small fraction over 1 cc. of fluid being displaced at the point of greatest arterial and venous pressures, and the return was, as far as venous pressure and fluid volume are concerned, exactly to the starting point at the same time, viz., 8 minutes after the injection. At that time the arterial pressure was 6 mm. lower than normal. It is thus perfectly clear that in this case following the injection of 2 cc. of the commercial extract of pituitary, the rise in arterial and venous pressures forced out 1 cc. of fluid into the bottle; this fluid rapidly made its way back into the canal during the period of readjustment of the vascular condition within the skull. New formation should have been possible in this case for the pressure was never appreciably increased (0.5 mm. of water) during the experiment and the return was not forced by any outside pressure. We therefore have made it clear that: The injection of extract of the pituitary is followed by the mechanical forcing out of the fluid from the canal during the rise in arterial and venous pressure, even if the fluid pressure is kept low this fluid returns to the canal in an amount exactly equal to that forced out during the pressor phase of action of the drug. We conclude: There is no increase in the amount of fluid in the canal during the action of pituitary extract, therefore, *pituiturin does not increase the rate of formation of the cerebrospinal fluid.*

Pilocarpine. As has already been stated, of all drugs having the power of stimulating the secretory mechanism of animals to activity, pilocarpine possesses this action to the highest degree. Thus if a

Fig. 3. The effect of pilocarpine sulphate on venous pressure and fluid volume. Note the remarkable similarity between the venous pressure and fluid volume curves.

secretory response on the part of the mechanism forming the fluid is to be expected in any case it is to be expected after pilocarpine. The changes in the venous and arterial pressure are sufficient to explain the changes in fluid pressure and in fluid outflow. In order to cover the ground more fully, we applied this additional method to the study of the action of pilocarpine upon the fluid. As a typical experiment we show figure 3. The time elapsing between the injection and the end of this record is almost exactly 15 minutes. The arterial curve is one typical of a small dose of pilocarpine, with a short period of inhibition and fall in arterial pressure, followed by escape of the beat from the vagus, followed by a rise in the arterial pressure. As can be seen from the graph the venous pressure in the skull rose sharply from the first, fell slightly during the arterial decline, then completed its rise, the maximum coming in 1 minute and 20 seconds, and then slowly fell exactly to normal (shown by the readings). The fluid volume curve is exactly similar, even the irregularities present on the venous curve being reproduced, except that the maximum came 40 seconds after the venous pressure maximum. The total fluid escaping was 1.3 cc. The fluid return also was practically exactly to the level normal before the injection of the drug. Thus it is perfectly obvious that we are dealing in this case with mechanical changes; the rise in venous pressure particularly forcing the fluid out into the bottle, only to permit it to return as the vascular condition of the skull returned to normal. Thus we conclude *small doses of pilocarpine do not increase the rate of formation of the fluid,* the increased outflow or the increased pressure is due to the alteration of arterial and venous pressure within the skull. Larger doses of the drug produce exactly the same effects. The fluid response in this case was small, although the venous pressure rose 69 mm. and the return of fluid to normal was immediate although the venous pressure remained high. When the vagus inhibition was released by atropine there was a temporary rise in venous pressure, a rise in arterial pressure and a prolonged increase in fluid volume forced out into the bottle, an increase persisting throughout the curve. *The fluid eventually all reëntered the canal.* This is undoubtedly the explanation of what happened in the cases reported by Dixon and Halliburton (2) who observed increased outflow following the injection of atropine. In these cases the fluid is forced out by alterations of pressure conditions within the skull, and would return during the period of readjustment. Frequently fluid return to the skull is hastened after atropine.

SUMMARY AND CONCLUSIONS

1. A method is described for measuring and recording graphically the amount of fluid in the skull under slightly less than the normal pressure.

2. Adrenalin does not increase fluid formation, because by this method it can be shown that the fluid flows out of the canal during the pressor stage of the action, but returns in equal amount while vascular readjustment is taking place.

3. In the same way it can be shown that pituiturin does not increase fluid formation.

4. In the same way it can be shown that pilocarpine does not increase fluid formation.

5. Atropine following pilocarpine may—but by no means always—produce increased arterial and venous pressure, accompanied by an increase in the amount of fluid forced into the bottle. The return of this fluid may be slow and tedious but finally takes place. It is believed that this also is a purely mechanical change not accompanied by new formation.

BIBLIOGRAPHY

(1) BECHT: This Journal, 1920, l, 1.
(2) DIXON AND HALLIBURTON: Journ. Physiol., 1913, xlvii, 215.

A STUDY OF THE INFLUENCE OF VARIOUS CIRCULATORY CONDITIONS ON THE REACTION TO THE LOW OXYGEN OF REBREATHING

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For the purpose of ascertaining whether certain conditions of the circulation may influence the ability for compensating to low oxygen, we selected from 2000 cases ten groups for special study. These selections include men with high and low systolic arterial pressures, high and low diastolic pressures, large and small pulse pressures, rapid and slow pulse rates, and men in whom the systolic pressure rose and others in whom it fell while they were standing.

For each group we have determined the character of the response to low oxygen by calculating the means, with probable errors, for 10 percentages of oxygen during the rebreathing experiment in which 52 liters of air were rebreathed until the subject became inefficient. The individual cases have been listed as to the final oxygen reached and whether the reaction was of the fainting or non-fainting type.

Low and high systolic arterial pressures. Assuming that a low systolic pressure may mean that the nutriment supply to the tissues is inadequate, and that a high systolic pressure, in that it is a measure of vascular and heart strain, might be associated with danger of heart strain during the low oxygen compensation, we selected for special study 26 cases in which the systolic pressure for the reclining posture was 98 mm. Hg. or less and 40 cases in which the reclining pressure was 138 mm. or more.

The compensatory reactions, as determined by calculating means, have been plotted for these two groups in figure 1. Several differences should be noted in the curves. On the whole the reactions are quite alike. However, toward the end of the rebreathing period there occurred in the diastolic pressure a more rapid and greater fall for the high than the low pressure group. The difference is best seen at 8 per

cent oxygen when the diastolic pressure had fallen 15.5 mm. for the high and only 5 mm. for the low systolic pressure group. In the high group the systolic pressure down to 8 per cent oxygen showed a gradual fall, dropping from 157 mm. at 3 minutes to 147 mm., while in the low group the systolic pressure maintained a level, being 114 mm. at 3 minutes and 113 mm. at 8 per cent oxygen. These differences in the systolic and diastolic pressures affect the pulse pressure which at 8 per cent oxygen had increased 16.3 mm. for the high as against 9.2 mm. for the low group.

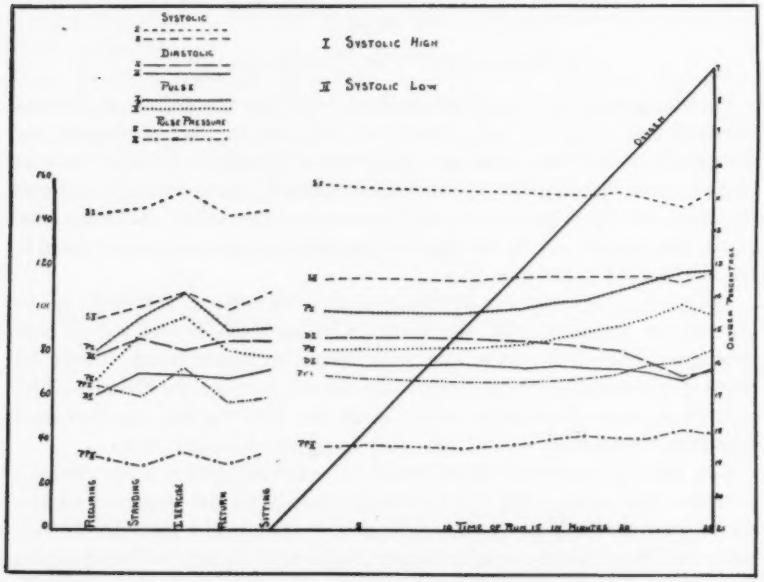

Fig. 1. Composite curves determined by calculating the means for the postural and low oxygen compensatory responses of two groups of men with high and low systolic pressures.

A comparison of the compensatory curves of these two groups with the normal curves of reaction presented in our earlier paper (1) shows that the changes followed the usual course in all factors, except the arterial systolic pressure. In the non-fainting type of response a slight or moderate rise in the systolic pressure occurs during the last 6 or 8 minutes. In neither of these two selected groups was the rise usually present. Seven, 17.5 per cent, of the 40 men with a high systolic pressure showed a terminal rise of from 2 to 10 mm.; while there were 8,

32.5 per cent, of the group of 26 low-pressure cases with a slight systolic rise toward the end. As a rule, therefore, men with a high systolic pressure make the compensation to low oxygen without further increasing the vascular strain by a compensatory rise. To prevent such an increase there occurs a greater vasodilatation than ordinary, as is evidenced by the diastolic pressure fall.

The question of increased strain has been further considered. Prior to the beginning of rebreathing, as the men were seated before the machine, the systolic pressure of the 40 men with a high pressure ranged from 134 to 160 mm. As has been shown only 7 had a final terminal rise, but all showed some degree of psychic rise which was maintained in part by the majority of the cases. So it might be assumed that the pressures met by the heart were extraordinary in some men. In this group the last determination of the systolic pressure, taken just before

TABLE 1

Summary of final oxygen reached

FAILED AT O_2 PER CENT	LOW SYSTOLIC PRESSURE GROUP				HIGH SYSTOLIC PRESSURE GROUP			
	Number of cases	Per cent of cases	Off by psychologist	Off by clinician	Number of cases	Per cent of cases	Off by psychologist	Off by clinician
10.9–10.0	1	3.9		1				
9.9– 9.0	0				3	7.5		3
8.9– 8.0	3	11.5	2	1	4	10.0	1	3
7.9– 7.0	15	57.7	13	2	21	52.5	11	10
6.9– 5.8	7	26.9	7		12	30.0	10	2
Total..	26	100.0	22	4	40	100.0	22	18

rebreathing was stopped, ranged between 110 and 182 mm. The highest pressures were as follows: 160–169 mm., 3 cases; 170–179 mm., 5 cases and 184 mm., 1 case. The man with a final pressure of 184 mm. went to exactly 6 per cent oxygen. He was 26 years old and his pressure was not above 170 mm. until the last 4 minutes of the test. The oldest man had just past 32 years. The pressures observed ought to be tolerated by young men for short periods of time without injury to the heart and blood vessels.

The percentage of oxygen at which the men of the two groups became inefficient or fainted has been tabulated in table 1. Approximately equal percentages of men with low and high systolic pressures went down to less than 8 per cent of oxygen. Hence it is evident that the height of the systolic pressure does not give an indication of how well a man may compensate to low oxygen.

The members of each group have been classified as to whether they belonged to the fainting or non-fainting type of reactors. Among those having a low systolic pressure 9, or 34.6 per cent, and among those with a high systolic pressure 11, or 27.5 per cent, belonged to the non-fainting type. Here again it is evident that the systolic pressure does not determine the type of reaction that will be made to low oxygen.

There were only 2 men in whom the systolic pressure remained at 100 mm. or less during the entire rebreathing experiment. They compensated to 7.4 and 7.9 per cent of oxygen respectively. The lowest systolic pressure observed in a seated subject was 93 mm. and this man reached 7.4 per cent oxygen. Available evidence indicates that the low systolic pressures here considered, keep the brain as well supplied with metabolism necessities during the conditions of the low oxygen of rebreathing as do higher systolic pressures.

Low and high diastolic pressures. Since it is the diastolic pressure that supplies the force that fills the coronary vessels of the heart, there must be a critical diastolic pressure below which the heart will suffer from a lack of an adequate supply of oxygen and nutriments. In order to determine the possible influence of extremely high and low pressures we have compared a small group of men in whom the reclining posture diastolic pressure ranged between 40 and 56 mm. and another in which it ranged between 90 and 98 mm. There appears to be no outstanding difference in the reactions of the two groups to the low oxygen of rebreathing. Failures from the development of the fainting reaction were more common among the men with a high than with a low diastolic pressure. For the six cases with the lowest diastolic pressure the final oxygen was 8.3, 8.1, 7.6, 7.4, 7.1 and 7.0 per cent respectively. None failed unusually early nor tolerated to an extremely low oxygen. Five of these men were removed by the psychologist at the appearance of complete mental inefficiency. It is evident, therefore, that as a rule the higher brain centers suffered from oxygen-want before the circulation was much affected. Hence it appears that the lowest diastolic pressure found among these aviators did not jeopardize the heart functions in the low oxygen test.

Low and high pulse pressures. Another circulatory measure of the nutritive stream to the tissues is the pulse pressure. Hence it appeared probable that with a low pulse pressure the brain, as well as other tissues, might be more quickly affected by the decrease in the oxygen of the rebreathing experiment than when the pulse pressure is high. We therefore selected 14 cases with the lowest and 19 with the highest pulse

pressure for a comparative study. The low pulse pressures ranged for the reclining posture between 26 and 32 mm., in standing between 20 and 28 mm.; and for the high between 62 and 84 mm. for both postures. By this scheme of selection men with low and high systolic pressures are again brought into contrast. The compensatory curves are therefore quite like those shown in figure 1.

In final oxygen reached by these men there were 37 per cent of the high pulse pressure group who went to 7 per cent or less, while in the low group only 14 per cent went as low. Nevertheless, it is found that when the individual cases are considered, no clear advantage is held by the man with a low or a high pulse pressure. A selection is given below of 5 cases with the lowest and 5 with the highest pulse pressure to illustrate this. The numbers give the pulse pressure at regular intervals throughout the test. The first number in each case is in millimeters of pulse pressure for the subject when seated just before rebreathing was begun. The second gives the pressure during the third minute of rebreathing and registers the psychic increase. The last 4 or 5 numbers bring out the low oxygen effect. The special cases follow:

		Per cent of final oxygen
(1)	25–30–28–30–28–29–30–32–36–30	7.6
(2)	28–46–40–32–31–30–32–36–32	7.9
(3)	31–36–38–36–38–37–33–32–40–52	6.2
(4)	31–42–38–36–40–40–38–44–46–62	7.9
(5)	33–30–32–24–28–30–30–36–34–32	7.3
(1)	73–104–88–80–82–82–92–108–112	8.2
(2)	71–106–104–106–101–100–100–102–122–136	7.4
(3)	71–76–66–70–76–80–72–80–96–88	7.4
(4)	68–80–82–83–80–83–86–90–106–108–112	6.9
(5)	67–70–69–67–62–64–65–64–68–70–74	6.8

There were about equal percentages of men in each group removed by the psychologist because of complete demoralization of voluntary attention and motor coördination. Hence it is evident that no material advantage is gained by either a high or low pulse pressure so far as the maintenance of the brain is concerned.

Rapid and slow rates of heartbeat. On the basis of the belief that a high heart rate indicates a poor physical condition and that physically fit men trained for muscular work show a slow rate, we selected two groups of men to determine what influence the heart rate has on the ability for compensating to low oxygen. There were 123 men with a rapid pulse whose rates were in the reclining posture 87 and above and

in the standing position 108 and above. In the other group with a slow pulse, there were 95 men whose rates in the reclining posture were 60 and less and when standing 75 and less.

The data for the two groups are summarized in the curves of figure 2. That selection on the basis of pulse rate differences does not isolate groups that differ in the vasomotor circulatory factors is well brought out in the arterial pressures for the several postures and during rebreathing. Throughout the entire period of rebreathing, the systolic, diastolic and pulse pressures were about equal and the changes almost identical for the two groups, and quite like those of the average run of cases.

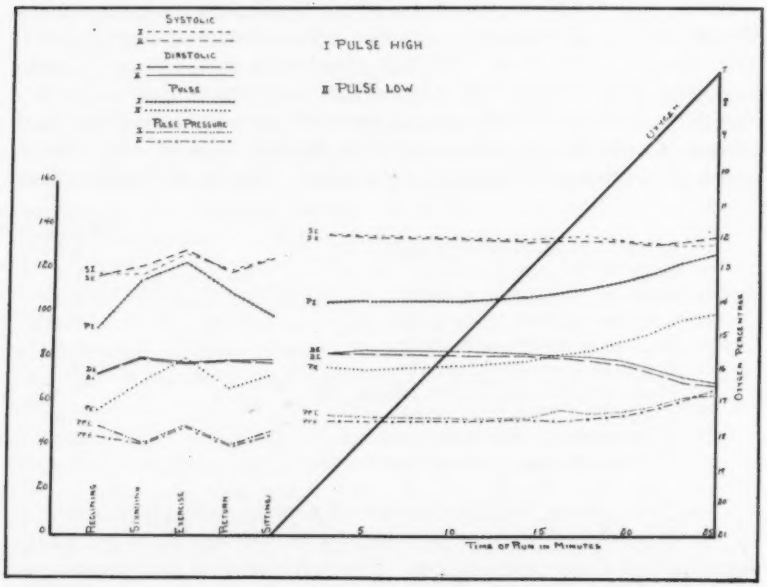

Fig. 2. Composite curves for a group of 123 men with a rapid and 95 men with a slow pulse rate.

The pulse rate curves are also similar, but on different levels. When sitting, just prior to the commencement of rebreathing, the rate for the rapid pulse group was 99 and for the slow 73; at 9, 8 and 7 per cent of oxygen during the rebreathing the increase in the number of beats in the pulse rate was for the former 20, 23 and 28 and for the latter 19,

25 and 28 beats respectively. A more equal degree of response could scarcely be expected on any other basis of choosing cases.

An inquiry to determine how low in oxygen the members of each group went brings out apparently a slight advantage in favor of the rapid pulse group. In this group 82 per cent of the men tolerated to less than 8 per cent oxygen while only 69.5 per cent with a slow pulse went as low. However, the two groups showed equal proportions of those who compensated to less than 7 per cent of oxygen. In the rapid rate group there were 46, or 37.4 per cent, and in the slow pulse rate group 35 cases, or 36.9 per cent, who went below 7 per cent oxygen.

All available evidence supports the conclusion that the initial pulse rate does not give an indication of the manner in which the response will be made nor of the capacity of the individual to respond to the low oxygen of the rebreathing experiment.

Rise and fall of the systolic arterial pressure on standing. It has been assumed that a fall in the systolic arterial pressure with standing gives evidence of physical weakness and unfitness. So we selected, from men who in the recumbent posture had a systolic pressure within the limits of 110 and 122 mm. Hg., 104 men, who showed on standing a compensatory systolic pressure rise of 12 mm. or more; and 117 who showed a fall of 8 mm. or more.

On this basis of selection two groups were obtained in which the mean pulse rates were almost equal and in which there were well-defined arterial blood pressure differences. However, beginning with these differences the two groups maintained them with surprising uniformity throughout the compensations to the low oxygen of rebreathing and showed up about equally well in the percentage of men who went to less than 7 per cent oxygen.

The mean value for the final oxygen percentage was calculated for each of our 10 groups. There was a striking uniformity in which all ranged between 7.2 and 7.7 per cent. The mean value for the final oxygen in a group of 2279 cases was 7.4 per cent. So we find that each of our 10 groups, selected because of a divergence from the average in some one circulatory factor, corresponded very closely to the majority of all men in ability to compensate to the low oxygen of rebreathing. The mean time taken by the 10 groups to reach the final oxygen ranged between 22.3 and 25.2 minutes. This also is close to the mean time found for 2279 cases, which was 24.7 minutes.

The mean increase in the per-minute volume of breathing for each of the groups was also very nearly the same, except in the high systolic

pressure group. This increase as registered for the last minute of rebreathing ranged between 3300 and 3700 cc. For the high systolic pressure group the mean respiratory increase was 4300 cc. per minute. These findings confirm our other data which show that the circulatory factor basis of selection does not separate men that differ in ability to compensate to low oxygen.

SUMMARY

Ten special groups were selected from 2000 cases for a study of the influence of various circulatory factors on the power of compensating to low oxygen. These included high and low systolic pressures, high and low diastolic pressures, large and small pulse pressures, rapid and slow pulse rates and cases of systolic pressure rise and of a fall on standing. All of the groups responded in a similar manner and compensated to equally low percentages of oxygen. None of the conditions studied placed the heart or the nervous system under a handicap greater than is present in average conditions of the several circulatory factors considered.

BIBLIOGRAPHY

(1) SCHNEIDER AND TRUESDELL: This Journal, 1921, lv, 223.

THE CORRELATION BETWEEN MOTOR CONTROL AND RIFLE SHOOTING

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While carrying out a series of student experiments with the steadiness test as described by Whipple,[1] it occurred to one of us (D) that involuntary movements or tremors probably play an important, if not a predominant part in rifle shooting. To test the truth of this hypothesis we carried out a short series of preliminary experiments with a group of five soldiers,[2] two sharpshooters (revolver) and three others who had failed to qualify as marksmen. These preliminary experiments encouraged us to carry out more extensive trials with large numbers of men and improved apparatus. With the coöperation of the office of the Surgeon General, we were able to test seventy-three men of known marksmanship at Camp Meade. In calculating the coefficient of correlation between rifle shooting ability and steadiness we used the rank difference method. This gave us a positive correlation of 0.61. Since this correlation is well within the significant and practically useful region, it may prove to be of value in selecting *a*, men physiologically unfitted to become rifle shots; and *b*, men who show an unusual degree of motor control and are therefore preferred material for intensive range training. The advantages of improving motor control and therefore shooting ability by practice with the steadiness apparatus rather than with firearms will be discussed further on.

Apparatus. Our preliminary experiments were carried out according to Whipple's directions (pp. 156–157), but we soon found several serious practical objections to this method. The Whipple apparatus consists of a brass plate set at an angle of 45° and pierced with two series of holes whose diameters, in sixty-fourths of an inch, are 32, 20,

[1] Whipple, G. M., 1914, Manual of mental and physical tests, Baltimore, Warwick & York, Inc.

[2] We are indebted to Col. O. G. Brown for placing these men at our disposal.

16 and 13 in the top row and 11, 10, 9,8 and 7 in the bottom row. A metallic needle (size not stated) with a flexible connection, is wired in series with the plate, two dry cells and a telegraph sounder or buzzer, in such a way that contact between the needle and plate will actuate the sounder. A writing lever attached to the armature of the sounder records contacts on a kymograph.

The subject is instructed to hold the needle within the largest hole and to maintain this position, so far as possible, without touching the brass plate during the 15 seconds allowed for the trial. The click of the sounder serves as a warning for him that the needle is making contact with the plate. This precaution is particularly necessary in the case of the smaller holes.

The chief objections to this method may be summarized as follows: *1*, The arrangement of the holes in two rows introduces a disproportionately large error in passing from the fourth hole at the end of the top row to the fifth at the beginning of the second row;[3] *2*, when the test is given in a standing position, there is no simple way to adjust the instrument to the varying heights of different subjects; *3*, the counting of the number of contacts on the kymograph is a laborious and time-consuming process, and, in the case of the smaller holes, the *duration* of a single contact introduces a complicating factor.

To overcome these objections to the Whipple method, we used an apparatus essentially like one devised by Dr. Knight Dunlap. Our instrument consisted of two circular brass plates, six inches in diameter, mounted, one-eighth of an inch apart, on the end of a half-inch brass rod ten inches long. The test holes were drilled through the outer plate which could be revolved so as to bring every hole into the same relative position in front of the subject and directly over a thirteen-sixteenths inch hole in the second plate. After dividing the plate into sixteen equal sectors, fifteen holes were drilled five-eighths of an inch from the edge of the plate as follows: 32, 28, 24, 22, 20, 18, 16, 14, 13, 12, 11, 10, 9, 8 and 7 sixty-fourths of an inch respectively. The second plate, containing only the $\frac{13}{16}$ inch hole, covered all the holes in the outer plate except the one into which the needle was inserted. The subject's attention could therefore be concentrated more easily on the test hole.

By means of clamps and a heavy vertical stand, the brass plates were adjusted to suit the heights of different subjects. The plates

[3] Personal communication from Dr. Knight Dunlap.

were set at an angle of 45° and, with the help of a horizontal rod, the top edge of the plate was always placed on a level with the subject's right shoulder. Preliminary trials had shown this adjustment to be satisfactory and quite uniform. Our stylus was made of a 6½-inch piece of number 39 B. and S. stub steel stock (diameter 0.0995 inch) mounted in a light, cylindrical, wooden handle, 3½ inches long and ½ inch in diameter. The brass plate, stylus, two dry cells and buzzer and a double telephone receiver were all wired in series in such a way that the stylus in making contact with the brass plate actuated both the buzzer and the telephone. The buzzer served to warn the subject of prolonged contact in the smaller holes. The brief contacts which failed to sound the buzzer were easily picked up by the operator through the telephone. In place of the kymograph recording device, we used a Veeder mechanical counter and the operator pressed the counter each time the telephone clicked. This simplified method of recording contacts saved an enormous amount of time.

Experimental procedure. The experiments at Camp Meade were carried out in the hospital laboratory.[4] The apparatus was set up on a solid table in such a way that the subject stood with his back to the window; but the plate and stylus were well illuminated. One operator managed the telephone, counter and stop-watch, while the other operator instructed the subjects, adjusted the plates to the proper heights, recorded the names of the subjects on special cards and controlled any tendency on the part of the subjects to withdraw the stylus or to brace the elbow against the body. General instructions were reduced to a minimum by having six men in the laboratory at the beginning of the experiment and, as each man finished his trial, a new man entered and took his place. The test itself is so simple that the men readily understood what was expected of them by watching the five men ahead go through the experiment. As each man took his place before the apparatus, he was shown the significance of the buzzer and he was also given a brief preliminary trial, usually in hole number 16. This was intended as a "shock absorber"[5] and generally served to reassure the subject. In conducting the tests the subject was always allowed 3 or 4 seconds for taking the position in each hole, since a certain amount of movement

[4] We are indebted to the military authorities of the Third Corps Area and at Camp Meade for their generous coöperation in this research.

[5] For this and other excellent practical suggestions regarding the giving of psychological tests see Link, H. C., 1919, Employment psychology, Macmillan Co.

appears when the needle is first inserted that is almost immediately checked by the subject's own control. The time for each trial was 20 seconds. We found no difficulty in giving the test at the rate of twenty men per hour.

Tests made with two groups of students during the past 2 years had shown that hole number 13 is a satisfactory starting point. In the majority of cases each man was therefore tested in holes 13, 12, 11 and 10. When the subject made a total of less than 50 contacts in these four holes he was also tested in number 9. Preliminary experience had shown that when the total number of contacts for any series of holes is greater than about 50 it is generally useless to make observations in the next smaller hole, since the contacts then tend to become so frequent that an accurate count is impossible. We therefore adopted this principle with all subjects. In six cases the subject could not get beyond hole number 11; and the most unsteady man, who was likewise the poorest marksman, made 88 contacts in hole number 13. (See table 3.) It is of interest that this individual was the only subject of more than one hundred tested who made more than 50 contacts in hole 13. This fact seems completely to justify our selection of hole 13 as a practical starting point for the test.

An analysis of rifle shooting. Before discussing our method of scoring the steadiness records and their correlation with marksmanship, a brief analysis of the physiological and psychological aspects of rifle shooting seems in order. Successful rifle shooting requires: *a*, vision sufficiently good to see the sights and target clearly and without blurring; *b*, a high degree of motor control (eye and hand coördination) to enable the marksman to hold the target, the front and the rear sights in alignment; *c*, a slow, steady pressure, not a jerk, on the trigger, the so-called "trigger-squeeze," applied at the moment of sight and target alignment; and *d*, a complete indifference to the sound of the shot, that is, an absence of so-called "gun-shyness" or "flinching." Such technical points as sight elevation and judgment in the matter of cross wind currents need not concern us in this discussion.

Of these four factors the first, vision, can in most cases be corrected with proper glasses. The trigger-squeeze is a matter of practice and presents no insurmountable physiological or psychological obstacle. A "gun-shy" individual, whether man or dog, is generally considered hopeless, since the response is ordinarily quite beyond voluntary control. Probably, in the case of man, a study and exposure of the origin of the gun-shyness would remove the difficulty far more rapidly than

attempting to accustom the individual to the sound of gunfire. As a practical problem, however, gun-shy individuals are relatively rare. In our study we found a single man who made an excellent steadiness record but failed to qualify as a marksman. (See number 66 in table 3, column 1. This individual has the largest D^2 value also.) We suspect this man of being gun-shy but we have no positive evidence for our suspicion.

There remains the factor of motor control. Although an individual with an oscillating gun barrel is at an obvious disadvantage on the rifle range, neither the degree of involuntary tremors nor the control of such tremors has ever received systematic study in the case of marksmen. In our opinion, involuntary movements constitute an important and easily measured barrier to successful marksmanship. It must remain for future investigations to show whether training of involuntary movements by the use of a steadiness apparatus will improve rifle shooting. The practical advantages of preparing men to become marksmen by laboratory training rather than by range practice are too obvious to require further comment.

The rating of marksmanship. Rifle shots in the Infantry of the United States Army are divided into six groups, as shown in the following table.

TABLE 1

GRADE	RIFLE FIRING POINTS	NUMBER OF MEN TESTED
1. Expert rifleman	253	3
2. Sharpshooter	238	17
3. Marksman	202	14
4. First-class man	177	12
5. Second-class man	152	10
6. Unqualified	Less than 152	17

Although all men in the first five grades must attain a high degree of accuracy in estimating distances, the main criterion for ranking rifle shots is actual range score. In our table 3 we have therefore ranked our seventy-three subjects on the basis of their best range scores, made under uniform and carefully controlled conditions. The exact details of distances, number of shots fired, method of scoring targets, etc., are irrelevant to our discussion but may be found in the Small Arms Firing Manual for 1913, issued by the War Department.

Method of scoring the steadiness test. In our laboratory experience with student subjects, we found that the steadiest individuals could hold

the stylus for 20 seconds in holes 13, 12 and 11 without a single contact. As an empirical basis for comparison, we therefore counted all contacts in hole number 10 as single units, but penalized contacts in 11, 12 and 13 by counting single contacts as 5, 10 and 20 units respectively. The

TABLE 2

Method of scoring the steadiness records

HOLE NOS.	CONTACTS		UNITS		SCORE
		Subject 1			
13	0	×	20.0	=	0
12	0	×	10.0	=	0
11	2	×	5.0	=	10
10	3	×	1.0	=	3
9	14	×	0.5	=	7
Total.....	19				$\frac{20}{5}=4$
		Subject 19			
13	2	×	20.0	=	40
12	3	×	10.0	=	30
11	10	×	5.0	=	50
10	22	×	1.0	=	22
9	38	×	0.5	=	19
Total.....	75				$\frac{161}{5}=32.2$
		Subject 47			
13	4	×	20.0	=	80
12	11	×	10.0	=	110
11	19	×	5.0	=	95
10	32	×	1.0	=	32
9					
Total.....	66				$\frac{317}{4}=79.2$

test was so difficult in hole number 9 that in this case two contacts were counted as one unit. The total number of units was then added and divided by the number of holes tested (3, 4 or 5) which gave a steadiness score for a comparative rating. The exact method of scoring with three records taken from our observations is shown in table 2.

TABLE 3

Correlation of marksmanship and steadiness by rank differences

MARKSMANSHIP		STEADINESS		D^2	MARKSMANSHIP		STEADINESS		D^2
Rank	Score	Rank	Score		Rank	Score	Rank	Score	
1.0	266	1	4.0	0.00	38.0	196	34	55.5	16.00
2.0	264	16	27.9	196.00	39.0	193	59	140.7	400.00
3.0	253	25	39.2	484.00	40.0	190	10.5	16.0	870.25
4.0	248	14	25.5	100.00	41.0	189	43	66.5	4.00
5.0	247	5	13.6	0.00	42.0	188	58	135.2	256.00
6.0	244	38	60.5	1024.00	43.0	186	50	88.2	49.00
7.0	242	37	58.6	900.00	44.0	182	66	195.0	484.00
8.0	241	8	15.1	0.00	45.0	179	30	44.5	225.00
9.0	240	60	143.5	2601.00	46.0	178	4	13.1	1764.00
11.0	239	19	32.2	64.00	47.0	176	56	118.2	81.00
11.0	239	12	17.3	1.00	48.0	173	54	108.0	36.00
11.0	239	36	58.4	625.00	49.0	171	31	49.2	324.00
16.5	238	6	14.4	110.25	50.0	169	63	164.0	169.00
16.5	238	21	34.2	20.25	51.0	168	42	66.2	81.00
16.5	238	13	18.3	12.25	52.0	163	62	153.2	100.00
16.5	238	39	61.7	506.25	53.0	158	67	240.0	196.00
16.5	238	15	27.5	2.25	54.0	156	46	76.5	64.00
16.5	238	22	36.5	30.25	55.0	154	35	57.5	400.00
16.5	238	41	65.2	600.25	56.0	153	55	111.5	1.00
16.5	238	2	7.9	210.25	57.0	145	68	270.2	121.00
21.0	230	49	83.2	784.00	58.0	140	51	94.7	49.00
22.0	229	29	44.4	49.00	59.0	137	64	164.5	25.00
23.5	225	32	50.2	72.25	60.0	135	40	64.8	400.00
23.5	225	28	41.5	20.25	61.0	133	17	30.5	1936.00
25.0	218	26	40.2	1.00	62.0	124	71	335.0	121.00
26.0	215	24	39.0	4.00	63.0	122	53	107.7	100.00
27.5	213	23	38.0	20.25	64.0	114	70	316.6	36.00
27.5	213	44	71.7	272.25	65.0	112	33	51.5	1024.00
29.5	209	9	15.6	420.25	66.0	100	10.5	16.0	3080.25
29.5	209	48	79.5	342.25	67.0	90	72	580.0	25.00
31.0	205	27	40.5	16.00	68.0	76	47	79.2	441.00
32.5	204	18	31.6	210.25	69.0	75	69	295.0	0.00
32.5	204	7	14.6	650.25	70.0	67	57	125.5	169.00
34.0	203	52	100.7	324.00	71.0	61	65	186.0	36.00
35.0	199	45	72.7	100.00	72.0	44	61	149.5	121.00
36.5	197	3	11.9	1122.25	73.0	26	73	1280.0	0.00
36.5	197	20	33.2	272.25					

$\Sigma D^2 = 25372.00$

For 73 cases $\rho = +0.61$

The coefficient of correlation between rifle shooting and steadiness. We were interested in determining the degree to which great steadiness and expert shooting ability tend to occur in the same individual. We therefore calculated our coefficient of correlation between rifle shooting and steadiness by the rank difference method.[6]

The formula:

$$\rho = 1 - \frac{6 \Sigma D^2}{n (n^2 - 1)}$$

gives us a value for $\rho = +0.61$. In addition to each man's rank, we have given the actual rifle and steadiness scores in our table 3.

Where several men had the same score as the three men who made 239 on the rifle range (number 11, table 3) they were all given the same rank (11) and in this instance rank numbers 10 and 12 were not assigned. This is the customary statistical procedure. Although the high positive value of the coefficient of correlation is well within the significant range, it must not be imagined that a single expert rifle shot will inevitably make a high steadiness score. Predictive tests are useful as short cuts in grouping large numbers of individuals. They are incomparably more efficient, provided the tests themselves have been properly tested, than the methods of trial and error and learning by experience.

CONCLUSIONS

From the foregoing experiments it is clear that steadiness is definitely associated ($\rho = +0.61$) with marksmanship in the case of men who have had range experience. The practical value of a test of this sort lies in its use as a predictive means of selecting probable expert rifle material and eliminating men who are physiologically unfitted to qualify as marksmen. Since the steadiness test requires only about 3 minutes per man, it would be far more efficient as regards time, cost of munitions and the nerves of men and officers to select material for elimination or range development by this test rather than by the slow and costly method of giving every man a try-out with the rifle. In our experiments we have, however, only tested the test on men of known ability. It remains for future work to show how extensively this test may be used with raw recruits. Range practice may increase a man's steadiness and, conversely, steadiness practice may improve a man's range score. Further experiments alone will tell.

[6] The tables published by the Scott Co., Philadelphia, in the Journal of Applied Psychology, iv, 115, greatly facilitate the computation of the coefficient of correlation by the rank difference method.

A NOTE ON THE RÔLE OF THE INTRINSIC PLEXUSES IN DETERMINING THE EFFECTS ON GASTRIC MOTILITY OF VAGUS STIMULATION

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In a previous report (1) attention was called to the fact that prolonged tonic contraction of the muscle of the turtle's stomach cannot be induced by electrical stimulation of the vagus nerve. It was also pointed out that by cutting the nerves and allowing the animals to survive for one to two days in crucial experiments no evidence is obtained of tonic stimulating action, but on the contrary hypertonic muscular activity frequently follows destruction of the medulla or cutting of both vagi, thus indicating a tonic inhibitory action of the medullary centers.

It is however a fact that a tetanizing induced current applied to the vagus nerve will cause a generalized contraction of the stomach with or without peristaltic waves. The peculiar fact that this contraction soon disappears although stimulation of the nerve be continued, suggested a study of the rôle of the intrinsic plexuses in relation to vagus stimulation. For this we have contrasted the effects of vagus stimulation on gastric activity with that of direct stimulation of gastric musculature.

Methods. To record the effects of vagus stimulation the turtles were decerebrated, the medulla oblongata being left intact, and a rubber balloon was put into the stomach. This was connected with a water manometer for graphic tracing. In addition to this an opening was made in the back of the shell on the left side to expose the stomach to direct observation. After such an opening the animal can still breathe through the remaining lung and asphyxia is avoided. For direct stimulation of the stomach platinum electrodes were placed directly on the stomach or a strip of gastric muscle was suspended in oxygenated Ringer's solution.

In the work on the dog a gastric fistula was made and after the wound was healed the animal was decerebrated according to Sherring-

ton's method. Graphic tracings were made 6 to 24 hours later, and the vagus stimulated without the necessity of anesthesia.

Results: 1. If the vagus nerve be stimulated with tetanizing induced shocks for a long time (15 minutes or more) the stomach at first contracts strongly and then relaxes at the same rate as follows a spontaneous contraction, although the stimulation is being continued. After a time a second weaker contraction may follow but the stomach soon relaxes and remains quiescent although the stimulation be continued. A sudden increase in intensity of stimulation at this stage may be followed by a contraction and then the stomach relaxes in spite of the

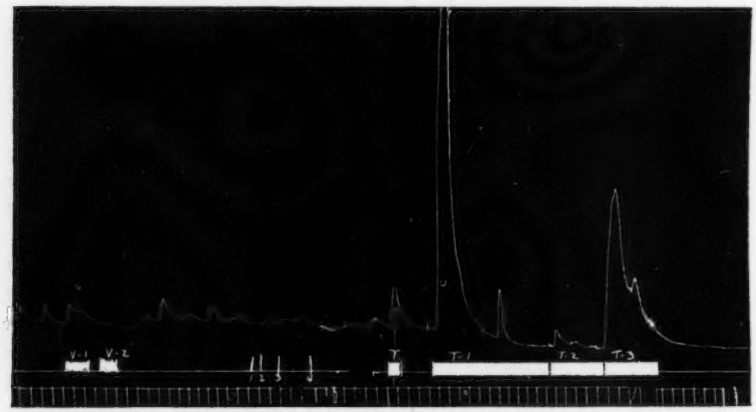

Fig. 1. Gastric contractions of decerebrate turtle. *V–1*, Passage of voltaic current through peripheral end of vagus nerve (6 volts); *V–2*, repeated with twelve volts; *1, 2, 3, 4*, stimulation of vagus nerve with voltaic current by single closing and breaking voltaic current; *T*, weak tetanizing induced shocks applied to the vagus; *T–1*, medium tetanizing shocks to vagus; *T–2*, very strong tetanizing current to vagus; *T–3*, tetanizing current directly to the stomach wall.

continued stimulation (fig. 1). The continuous application of the induced shocks to the vagus does not therefore lead to long-continued increase of muscle tone. On the contrary, after strong contractions the tonus is for a long time lower than that preceding the stimulation. This same fact has been noted in the dog (fig. 2). It seems to be a general rule that maximum contractions induced by vagus stimulation leave the stomach in a state of temporary depression.

2. Continuous passage of galvanic current through the vagus nerve gives no evidence of stimulating effects but sometimes there is a slight inhibitory effect. A series of voltaic stimuli given at the rate of six per second gave the same effects as induced shocks.

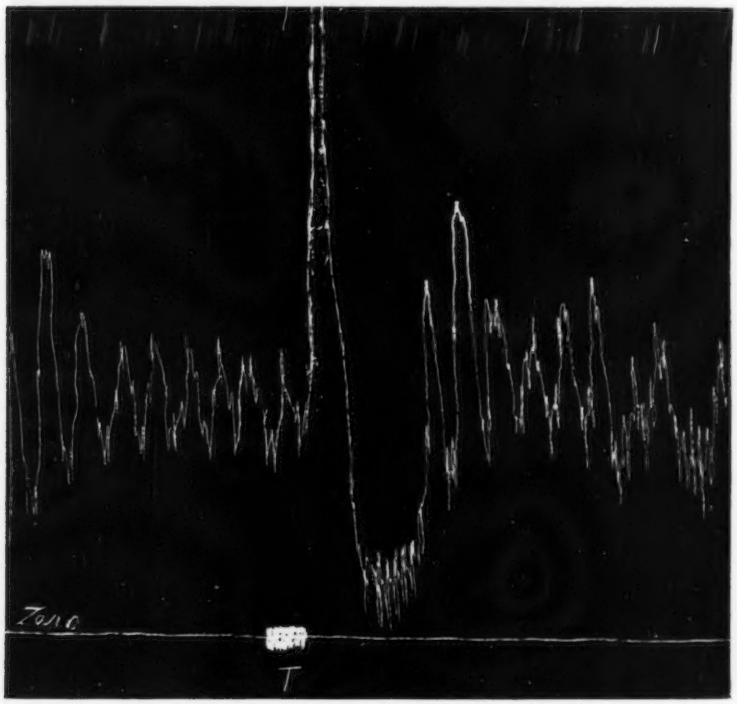

Fig. 2. Weak hunger contractions of decerebrate dog. Induced shocks on peripheral end of vagus. Contraction followed by inhibition.

3. Number of stimuli: A single induced shock, howsoever intense, applied to the vagus nerve, has never in our observation caused a gastric contraction.

4. Rate of stimulation: Single induced shocks at the rate of one every 2 seconds or slower leads to inhibition of gastric tone in the turtle. Single induced shocks at the rate of six per second or faster, lead to contraction. Mechanical stimulation such as handling the nerve or

traction on the nerve leads to inhibition in both turtle and dog (fig. 3). Weak stimulation or a slow rate of stimulation favors inhibition.

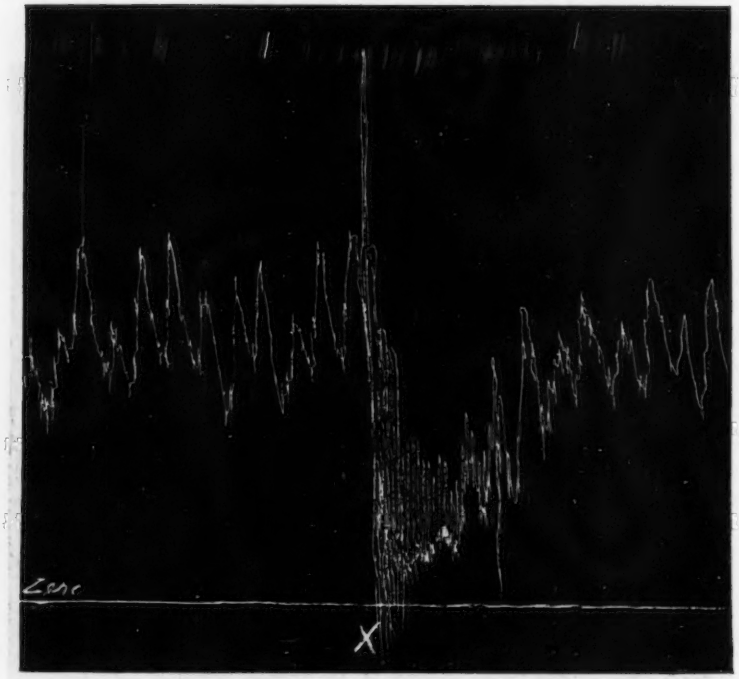

Fig. 3. Hunger contractions of decerebrate dog. *X*, Mechanical stimulation of peripheral end of vagus by traction on ligature.

5. Intensity of stimulation: The greater the intensity of the induced current, the less is the minimum number of induced shocks required to cause gastric contractions.

INTENSITY OF CURRENT	RATE OF STIMULATION	NUMBER OF SHOCKS	RESPONSE
9	6 per second	19	Minimum contraction
8	6 per second	15	Minimum contraction
7	6 per second	10	Minimum contraction
6	6 per second	5	Minimum contraction

Intensity measured on the arbitrary scale of the Harvard inductorium. Intensity "9" was barely susceptible to the tongue and "6" was painful to the tongue.

6. Influence of asphyxia: Prolonged asphyxia (12 to 48 hours) abolished the response of gastric contraction to vagus stimulation. Although when this stage was reached stimulation of the gastric musculature was followed by contraction.

7. Influence of temperature: If the temperature of the turtle is lowered to about 10°C., motor responses to vagus stimulation cease. Increasing the body temperature of the turtle up to 25°C. leads to increased vigor of gastric contraction after vagus stimulation. This temperature effect may of course be either a muscular or a neuromuscular affair.

8. Artificial rhythm: We have not found it possible to produce rhythmical contractions of approximately uniform extent by repeated periods of vagus stimulation.

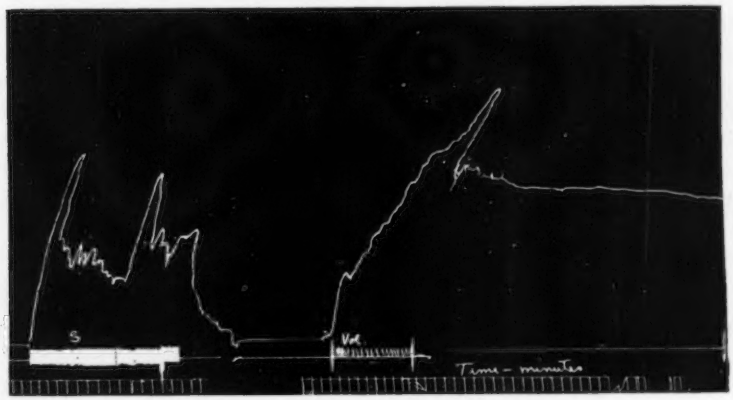

Fig. 4. Isolated strip of muscle of turtle stomach suspended in oxygenated Ringer's solution. *S*, Induced shocks applied directly to the strip; *Vol.*, passage of voltaic current through the isolated muscle strip.

These characteristic effects on the turtle stomach of vagus stimulation are not due to temporary asphyxia of the stomach due to simultaneous inhibition of the heart, for it requires hours of asphyxia to abolish vagus action, and these effects described can be induced for a time after ligation of the arteries from the heart. Furthermore, none of these are directly dependent on possible simultaneous inhibitory influences exerted through the splanchnic nerves for they can be elicited in the dog after cutting the splanchnic nerves and in the turtle after destruction of the spinal cord.

In contrast to the preceding, if the muscle strip be directly stimulated the following effects may be obtained:

1. Induced shocks at a rapid rate (6 to 40 per second) are followed by a contraction which persists as long as stimulation is continued (at least 15 minutes) (fig. 4).

2. By slow rate of stimulation we have not succeeded in inducing relaxation.

3. Continuous passage of a voltaic current leads to a tetanic contraction of the stomach which may continue during the flow of the current and also for an hour or more after the stimulating current is removed (fig. 4).

4. A single shock has given us no muscular contraction.

5. The muscle strip is much more resistant to asphyxia. Thus after the animal has been asphyxiated until stimulation of the vagus no longer induces contraction, on direct stimulation of the muscle itself, contractions can be obtained.

6. When the body temperature has been lowered until motor responses fail from vagus stimulation, direct stimulation of the muscle is followed by contraction.

7. An artificial rhythm is easily induced in the muscle strip by repeated short intervals of stimulation with the induced current.

DISCUSSION

From these experiments it is evident that any theory of the tonic action of the vagus on gastric motility must take into account the following considerations:

1. The vagus may have either an excitatory effect on gastric motility, or an inhibitory influence on gastric tone and contractions, in both dog and turtle, and according to Langley (2) and to Page May (3) the same is true of the rabbit, cat and monkey.

2. In the turtle no method of increasing gastric tonus is available by stimulation of the vagus.

3. Inability to induce a tetanic contraction in the turtle by vagus stimulation is dependent on the production of fatigue at the vagus synapses or refractory condition within the gastric nerve plexuses, and not to a peculiar condition of the muscle itself.

4. Lowering of temperature and asphyxia abolish the response of contraction to vagus stimulation, by acting on some part of the intrinsic plexuses rather than on the muscle tissue.

5. The inhibitory effects of vagus stimulation are not so susceptible to fatigue as the motor effects.

Page May has pointed out that no evidence of a tonic vagus effect is available in anesthetized mammals. Carlson (5) speaks of the inability to reflexly augment gastric tone or motility. As pointed out in this paper, relatively intense stimulation is required to overcome the resistance to conduction of the intrinsic plexuses. Drugs which are supposed to stimulate the medullary centers (picrotoxin and apomorphine) in the dog and rabbit have given only inhibition, and in the turtle temporary stimulation followed by depression (6). These considerations therefore raise the question as to whether or not the vagus normally exerts a tonic stimulating action on gastric motility. On the other hand, almost incontrovertible are the facts that stimulation of the vagus with the ordinary tetanizing induced shocks induces a short period of contractions, and the observations of Cannon (4) and Carlson (5) that sectioning both nerves leads to depression of gastric tonus. The only possible criticisms of the later results might be that there is no proof of nervous stimulation in the normal animal as intense as is required experimentally to cause contractions, and that depression of tonus after vagotomy may be secondary to some other disturbance in the viscera, perhaps sphincters, secretions, etc. The explanation of the paradox involved is to be sought perhaps in the further study of the properties of the intrinsic plexuses and conduction between the vagus and these plexuses.

BIBLIOGRAPHY

(1) Bercovitz and Rogers: This Journal, 1921, lv, 323.
(2) Langley: Journ. Physiol., 1898, xxiii, 407.
(3) Page May: Journ. Physiol., 1907, xxxi, 31; Brit. Med. Journ., 1902, xi, 779.
(4) Cannon: Mechanical factors of digestion, New York, 1911.
(5) Carlson: Control of hunger in health and disease, Chicago, 1916.
(6) Rogers: This Journal, 1918, xlv, 553.

COMPARATIVE STUDY OF ETHANOL, CAFFEINE AND NICOTINE ON THE BEHAVIOR OF RATS IN A MAZE

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The influence of drugs on psychological functions and on animal behavior has been very little studied by either psychologists or pharmacologists. The senior author in conjunction with several collaborators has found it convenient and productive of valuable results to study the pharmacological effects of drugs in this respect on albino rats in the circular maze. This apparatus enables the investigator to study not only the neuromuscular coördination of the animals, but also such specifically psychological phenomena as rate of learning, memory-habit and general behavior of the animals. Furthermore, in case of drugs, the maze offers, in the opinion of the authors, a convenient and valuable method of detecting the earliest and mildest symptoms of narcosis, using the latter term in its broadest sense, namely, as denoting a loss of normal responsiveness and automatic activity of the living organism. In this way Macht and Mora (1) have investigated the effects of opium alkaloids on albino rats; and again Macht and Bloom have made a similar study of the antipyretics, in one research (2), and have analyzed the cerebral effects of cocaine in another (3). In the present communication the authors propose to describe the results of a comparative study of three drugs which play an important rôle, not only in pharmacology, but also in social and personal hygiene—ethanol, caffeine and nicotine.

Description of the maze. A full description of the maze with illustrations has been published elsewhere (1), (2). Here only a brief recapitulation is made. The circular maze is made with wooden base and aluminum walls. The base is 150 cm. in diameter and 4 cm. in thickness. Its upper surface is marked off by grooves into a series of concentric circles. The diameter of each of the circles is as follows, beginning with the outermost one: 140 cm., 100 cm., 80 cm., 60 cm., 40 cm. and 20 cm. Into the circular grooves are inserted sheets of aluminum

18.5 cm. high and 0.8 mm. thick. Each strip of aluminum is cut just 10 cm. shorter than the length of the circular groove into which it is to be fitted, thus giving an opening into the alley. By means of this arrangement it is possible to slide the aluminum around in its groove and thus place the entrance in any desirable position. In the present investigation the 7 openings or entrances to the alleys were placed in such a position that the rat had to make alternate turns to right and left before reaching the center of the maze. In addition to the doors or openings, the alleys were provided also with obstructing partitions which formed a number of blind cul-de-sacs. A wire screen prevents the animals from crawling over the top. A camera lucida attachment to the maze has been invented by Professor Watson and is also described in full in the previous papers by the senior author. By means of this camera lucida attachment, the movements of an animal in the maze can be traced upon white paper with a soft pencil. Such tracings are especially useful in the study of the learning of the maze problem, such as has been done by Miss Hubbert and others. In the present investigation, where the effect of drugs on the behavior of the animals was studied after the rats had been trained, the use of this attachment was not essential and it was therefore dispensed with in a great many of the experiments.

The study of the behavior of the rats in the circular maze is begun by placing an animal in the center of the maze and feeding it for three successive days. During these three preliminary feedings, which last from 10 to 15 minutes, the entrance is blocked off so that the animal may not roam around. On the fourth day the rat is placed in the cage, then the trap-door is raised and the animal allowed to enter the first alley. The animal then gradually learns to find its way to the center of the maze, when it is taken out and the experiment is repeated. Generally three trials are made on each day. For work with the maze albino rats, which are tame, must be employed. The animals must be handled gently with the hands and under no circumstances must they be picked up with forceps or similar instruments. The most suitable animals are found to be rats approximately 60 to 90 days old. Older animals are apt to be sluggish, while very young rats do not learn the maze problem so readily. Ordinarily the albino rats learn the maze problem in about 2 weeks, and sometimes within a shorter period of time. An animal is considered to have solved the maze problem when it has learned to find its way into the center of the maze by the shortest route, that is, without any errors, on three successive trials. The technic of training is described in detail by Hubbert (4).

Analysis of the data furnished by the maze. In studying the effect of drugs, the maze problem can be utilized in two ways. Animals may be subjected to the influence of the drug action first and then trained in the maze with the purpose of ascertaining the effect on the rate of learning. Again, animals may be first taught to solve the maze problem and then the effect of a drug is studied in reference to its influence on their behavior, memory-habit, etc. Furthermore, other data can be obtained from the maze, after administering drugs to rats, which show the effect on neuromuscular coördination and various somatic changes. As to exactly what the mechanism of learning the maze problem may be, the explanations given by various experimental psychologists differ widely. Among the hypotheses which have been advanced to account for the reintegration of conduction paths in learning, there are at least three which stand out as rather opposed to one another in respect to the neural processes which they imply (5). The hypothesis suggested by Ladd and Woodworth (6) assumes inhibition of successive activities as the fundamental process which results in the selection and fixation of random activities. The second hypothesis, given by Angell and others (7), assumes nervous reinforcement as the fundamental process by which successive acts become linked together in habit-formation. The third hypothesis, that of Watson (8), depends chiefly upon the chance spreading of nervous excitation, or the simultaneous activation of two afferent pathways in such a way that the final common part of one is able to divert the discharge of the other and so bring about a permanent connection between itself and this afferent path. These hypotheses by no means exhaust the theoretical considerations of the maze problem (9). For the study of drug action, however, the various theoretical considerations are of secondary importance and the data obtained are of a much more definite nature, as will be seen from the following exposition.

Experimental data. In the present investigation a total of 40 rats was used. Most of the animals were males from 2 to 6 months old, a few females were also used. The drugs studied were dissolved in sodium chloride solution of 0.8 per cent and given by injection immediately after three normal trials were made in the maze. At the expiration of about 30 minutes the effects of the drugs were investigated and additional readings were made one or more hours later. Wherever possible the effects of the three drugs were studied on the same individuals, though, of course, not on the same days.

Effect of ethanol. Two to 4 per cent solutions of absolute ethanol in saline were employed. These were injected in some cases intraperitoneally and in other cases intramuscularly. No difference in the effects was noted between the two methods of administration. Control experiments were made with saline solution on the one hand and with distilled water on the other. The most striking feature in connection with the ethanol action noted, was its comparative low toxicity. One, 2, 3 and even more cubic centimeters of a 2 per cent solution

TABLE 1

Effect of ethanol

EXPERIMENT	DOSE PER 100 GRAM WEIGHT	NORMAL		THIRTY MINUTES		THREE HOURS		EFFECT
		Time	Error	Time	Error	Time	Error	
	gm.							
I	4.0	38	1	29	0	25	0	No effect
II	10.5	13	0	14	0	14	0	No effect
III	12.0	20	0	19	0	18	0	No effect
IV	12.0	17	0	26	0	14	0	Doubtful effect
V	12.0	23	0	16	0	16	0	No effect
VI	13.0	21	0	20	0	20	0	No effect
VII	16.0	15	0	16	0	15	0	No effect
VIII	25.0	31	0	20	0	28	0	No effect
IX	32.0	28	1	26	1	24	1	No effect
X	36.0	16	1	15	0	15	0	No effect
XI	52.0	14	0	20	0	15	0	Doubtful effect
XII	65.0	16	0	23	1	34	0	Depression
XIII	65.0	20	0	23	0	34	1	Depression
XIV	80.0	17	0	19	0	19	0	Slight depression
XV	80.0	23	0	25	1			Depression
XVI	90.0	22	0	22	0	27	0	Depression
XVII	95.0	21	0	39	1			Depression
XVIII	95.0	14	0	102	2	33	1	Marked depression
XIX	105.0	16	0	85	5	35	2	Marked depression
XX	125.0	22	1	63	4			Marked depression

were found to produce no appreciable effect on the rats as far as could be ascertained from their behavior in the maze. The smallest doses of ethanol which were found to produce a change were from 60 to 65 mgm. by weight of the drug. This effect was indicated by a slowness of progression, slight neuromuscular incoördination and disturbances in memory-habit as shown by errors committed. Table 1 expresses some of the data obtained with this drug. No primary improved or stimulation effect in running time or discrimination was noted after injections of ethanol in any of the experiments.

Effect of nicotine. Nicotine tartrate was dissolved in normal saline solution. This was injected either intraperitoneally or intramuscularly. The drug was found to be much more depressant than either ethanol or caffeine. The smallest dose to produce any effect was found to be 0.02 mgm. Such a dose of the tartrate is equivalent to about 0.0066 mgm. of the alkaloid nicotine itself. The injection was found to produce depression in the behavior of the animals as indicated by slower progression and failure in discrimination and memory. In no case was there any primary stimulation noted. Another striking feature of the action of nicotine was seen in the presistence or duration of the effects. Distinct impairment in the psychological functions of the rats was generally noted on the following day and even later. Table 2 indicates some of the results obtained with nicotine.

TABLE 2

Effect of nicotine tartrate

EXPERIMENT	DOSE PER 100 GRAM WEIGHT	NORMAL		THIRTY MINUTES		THREE HOURS		EFFECT
		Time	Error	Time	Error	Time	Error	
I	0.010	38	0	35	0	35	0	No effect
II	0.015	26	0	27	0			No effect
III	0.015	27	0	18	0	20	0	No effect
IV	0.016	19	0	22	0			Doubtful effect
V	0.02	18	0	49	0	56	0	Depression for 2 days
VI	0.02	15	0	35	2	20	3	Depression
VII	0.02	29	0	38	1			Depression
VIII	0.02	27	0	29	1			Depression
IX	0.025	18	0	26	0			Depression
X	0.025	15	0	12	0	21	0	Depression on next day
XI	0.03	17	0	19	0	Stalled		Depression
XII	0.03	44	0	80	3			Depression
XIII	0.03	21	0	30	1			Depression
XIV	0.05	13	0	72	2			Depression
XV	0.05	22	0	15	0	16	0	Depression on next day
XVI	0.06	17	0	16	0	16	0	Depression on next day
XVII	0.06	30	0	40	1	70	4	Depression for 2 days
XVIII	0.06	20	0	124	3			Marked depression
XIX	0.075	62	0	Stalled		Stalled		Marked depression for 2 days
XX	0.100	22	0	43	2	31	0	Depression

TABLE 3

Effect of caffeine

EXPERIMENT	DOSE PER 100 GRAM WEIGHT	NORMAL		THIRTY MINUTES		THREE HOURS		EFFECT
		Time	Error	Time	Error	Time	Error	
I	0.06	20	0	21	0	21	0	No effect
II	0.10	22	0	21	0	24	0	No effect
III	0.12	13	0	16	0	15	0	No effect
IV	0.12	18	0	41	0	23	0	Slight depression
V	0.30	15	0	15	0	15	0	No effect
VI	1.00	18	0	17	0	20	0	Slight depression
VII	1.00	18	0	17	0	17	0	No effect
VIII	1.25	15	0	14	0	15	0	No effect
IX	1.60	15	0	14	0	16	0	No effect
X	2.50	19	0	19	0			No effect
XI	3.00	18	1	18	1	18	0	Jerky movements
XII	4.00	15	0	16	0	16	0	Jerky movements
XIII	5.00	15	0	30	1	16	0	Depression
XIV	5.00	15	0	16	0	16	0	Doubtful effect
XV	5.00	51	1	47	1	29	0	No effect
XVI	6.00	20	0	30	1			Depression
XVII	6.50	18	0	20	0	20	0	Depression
XVIII	7.00	24	0	24	0	17	0	No effect
XIX	7.50	33	0	36	2			Depression
XX	7.50	15	0	70	4			Great depression Straub P.
XXI	8.00	18	0	28	1			Depression
XXII	8.00	25	0	19	0			No effect
XXIII	8.50	15	0	15	0			No effect
XXIV	9.00	17	0	22	0			Marked depression
XXV	10.00	20	0	20	0			No effect
XXVI	10.00	17	0	27	1			Marked depression
XXVII	13.00	15	0	Stalled		Stalled		Great depression for 2 days. Straub P.
XXVIII	14.00	27	0	Stalled		Stalled		Great depression. Diuresis
XXIX	15.00	16	0	Stalled		Stalled		Straub P. Death after 2 days
XXX	15.00	15	0	Stalled		Stalled		Straub P. Slow recovery after 5 days
XXXI	25.00	17	0	Dies				
XXXII	30.00	16		Dies				

Effect of caffeine. The drug employed was the alkaloid caffeina U. S. P. This was dissolved in saline to make solutions of 0.2 per cent and 0.5 per cent and the drug was in this case also injected either intraperitoneally or subcutaneously. In the present investigation the authors noted no primary improvement or any stimulation effect after injection of the drug as indicated by a shortening of the running time or by a lower number of errors. Occasionally there was noted, however, a nervousness or excitability of the animals as indicated by a jerkiness or suddenness in their movements. When studied in the maze however the effect on the animals, even in such cases, could not be called a stimulating one, but rather of a depressant character. The smallest doses of the drug to produce any change were 5 to 6 mgm. per 100 grams of weight. Such doses were found to retard the progression of the animals and produce a depression in their memory-habit, as may be seen from table 3.

DISCUSSION

A comparative study of all the data obtained by the authors with the three drugs examined reveals the fact that as far as the effects on the intelligent behavior of rats in the maze are concerned they are all depressant, that is, when given in sufficient quantity they impair the running time of the animals and the accuracy of discrimination or choosing the proper path or so-called memory-habit. The authors were not able to discern in any case, even after very small doses of the drugs examined, any stimulating effect in this respect. The only drug of the three in question, as far as they know, that has been experimented with in the maze, was caffeine. Lashley (5) investigated the effects of small doses of caffeine when injected into rats on their behavior in the maze: and his results show also that this drug does not produce a real stimulation.

It is interesting to note that of the three substances studied by the present authors, ethanol is much the least toxic. Large doses of this drug must be injected in order to produce a depressant effect. The smallest dose giving a definite depression was found to be 80 mgm. per 100 grams weight of the animals; although in some individuals doses of 60 mgm. also suggested a slight depressant action. Nicotine was by far the most toxic of the three drugs. It was interesting to note however that the effects of this poison did not manifest themselves always immediately after injection. The depression came on sometimes much later and in almost all cases persisted for at least 2 days.

The effects of caffeine were almost as unexpected as those of ethanol. Whenever this drug was found to produce an effect, such effect was of a depressant character. The smallest doses found to be effective in this way were 4 or 5 mgm. per 100 grams weight of the rats. Such doses while never producing an improvement in the absolute running time of the animals, or in their accuracy, often produced a spasmodic jerkiness in the muscular movements of the animals, so that the animals would start off to run at a very rapid gait. Such movements, however, were generally combined with an increased number of errors, so that the total time consumed in reaching the center of the maze was prolonged. After large doses of caffeine the impairment in the behavior of the rats was much more marked, but in the case of this drug, unlike the experiences with the other two drugs, there was a greater number of individual variations. It would seem that young rats were more sensitive to the action of the drug than older ones, but this observation requires further corroboration. After large doses of caffeine such as 10 to 20 mgm. per 100 grams weight, the impairment in the neuromuscular functions and the cerebral response of the rats lasted for many days, sometimes for 2 weeks. It was further noted that after large doses of caffeine the rats exhibited a stiffening of the tail, that is, the so-called Straub phenomenon. This was not surprising inasmuch as the analysis of the mechanism of this phenomenon shows that it is produced by a muscular spasm of the sphincters of the bladder and the rectum (10).

A comparison of the relative toxicity of ethanol, caffeine and nicotine obtained in the present investigation with the rats naturally invites a comparison of the relative toxicity of these drugs in man. Thus it would be interesting to determine the relative quantities of the three pharmacological agents, ethanol, caffeine and nicotine, which are absorbed by man on imbibing various beverages or on smoking cigars and tobacco in other forms, on the one hand, and comparing the relative toxicity of the same drugs for man, on the other hand. Such a discussion is however beyond the scope and purpose of the present paper.

SUMMARY

1. The effects of ethanol, caffeine and nicotine were studied on the behavior of rats in the circular maze.

2. It was found that none of the drugs studied by this method produced an improvement or stimulation in the behavior of the rats.

3. It was found that all of the three drugs when injected in sufficient quantities produced a depressant effect, nicotine tartrate being the most toxic, caffeine coming next and ethanol being the weakest in this respect.

BIBLIOGRAPHY

(1) MACHT AND MORA: Journ. Pharm Exper. Therap., 1920, xvi, 3.
(2) MACHT AND BLOOM: Journ. Pharm. Exper. Therap., in press.
(3) MACHT AND BLOOM: Arch. Internat. de Pharm. et Therap., 1920, xxv, 8.
(4) HUBBERT: Journ. Animal Behavior, 1914, iv, 60.
(5) LASHLEY: Psychobiology, 1917, i, 141.
(6) LADD AND WOODWORTH: Elements of physiological psychology, 551.
(7) ANGELL: Psychology, 4th ed., New York, 1909, 70.
(8) WATSON: Behavior: An introduction to comparative psychology, New York, 1914.
(9) DASHIELL: Psychobiology, 1920, ii.
(10) MACHT: Proc. Soc. Exper. Biol. and Med., 1920, xvii, 100.

THE MODIFICATION OF THE CROSSED EXTENSION REFLEX BY LIGHT ETHERIZATION AND ITS BEARING ON THE DUAL NATURE OF SPINAL REFLEX INNERVATION

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In the course of a class demonstration on reciprocal innervation in the decerebrate cat an interesting effect of light etherization on reflex behavior was recently noted; it appeared to be of such significance in its bearing on the physiology of the spinal nerve centers as to warrant further study.

The normal dominant reflex responses to stimulation of an afferent nerve in a hind limb are the flexion reflex and the crossed extension reflex; the flexion reflex consists in reflex excitation of the flexor muscles and inhibition of the extensor muscles in the same limb as the stimulated nerve; the crossed extension reflex consists in reflex excitation of the extensors and inhibition of the flexors in the opposite hind limb (1, p. 108). Many researches have pointed to the view that in general the stimulation of an afferent nerve tends to provoke two antagonistic central effects. Thus when an afferent nerve in a hind limb is stimulated the effect is to produce not simply the dominant reflex responses just described, but also a tendency in an exactly opposite sense which may or may not be latent (2); that is, a tendency to produce excitation of extensors and inhibition of flexors on the same side as the stimulated nerve. Sherrington and Sowton (3) using a decerebrate cat, showed that whereas the usual and universally obtainable result of stimulating an afferent nerve in the hind limb was to produce reflex inhibition of the knee extensor, vasto-crureus, they could under certain conditions change this result into the direct opposite, namely, reflex excitation of the extensor muscles, by changing the strength and form of the electrical stimulus, provided the extensor muscles exhibited at the outset a fair degree of reflex tonus. Strong faradization regularly produced inhibition; weak faradization sometimes produced excitation, and weak alternating currents with a frequency of 20 cycles a second and con-

sequently much less abrupt onset than induction shocks, were especially efficacious in producing the excitatory response. In short, strong and abrupt stimuli regularly provoked inhibition; weak stimuli, especially consisting of currents with gradual onset, provoked reflex excitation, if a sufficient background of reflex tonus existed.

Sherrington has investigated the phenomenon of extensor rebound following inhibition (4). This is present in a large proportion of decerebrate preparations, and consists of a fairly vigorous reflex contraction of extensor muscles following the cessation of stimulation applied to an afferent nerve in the same limb, when their tonic contraction has been inhibited during the application of the stimulus. This response was first likened to the setting free of a pent up stream of energy (1, pp. 203–212), but after Sherrington and Sowton had found that an immediate excitatory effect was being produced by afferent stimulation, replacing under certain conditions the usual inhibitory effect, they proposed an explanation of this rebound contraction as being due to a twofold reflex influence exerted by the stimulus during its application, and the greater persistence of the excitatory effect after its withdrawal (5). Sherrington's observations on the rebound contraction include additional reasons for this view, to which attention has already been called (6, p. 160). It may well be emphasized that the flexion reflex is notably characterized by its brief latency and brief after-discharge, in short, by its approximate limitation to the duration of the applied stimulus. The crossed extension reflex on the other hand is strikingly characterized by a longer latency and a very much longer after-discharge (1, p. 77). One of its most salient features is the fact that it outlasts the exciting stimulus usually by many seconds. It is also well established that the flexion reflex is usually much the more easily provoked of the two, and if with equally strong stimuli an attempt is made to provoke the two simultaneously the flexion reflex normally completely dominates in the reflex effect. Therefore if in an afferent stimulus a tendency to produce a central effect similar to the crossed extension reflex were to exist together with a tendency to produce the flexion reflex, we should expect exactly what we find in the usual response to stimulation of a single afferent nerve in the limb under observation; namely, flexion during application of the stimulus and, following its cessation, the appearance of the extensor effect which is well known to be characterized by its after-discharge or tendency to outlast the stimulus.

Graham Brown (7) has reported a rhythmic reflex response to simultaneous application of two antagonistic stimuli; that is, to stimulation

of afferent nerves in both hind legs. At the same time I reported a similar but more rapid rhythm in response to the same procedure (8). In certain preparations I found a similar rhythmic response to the stimulation of a single afferent nerve, and was led to regard this as evidence of the two antagonistic elements in the central effect. Sherrington (9) found that in the cat or dog a small dose of strychnine converted the usual inhibitory effect on the knee extensor of stimulating an afferent nerve, into an excitatory effect. He found that this change could then be undone by administration of chloroform or ether. Subsequently Sherrington and Sowton (10) reported that in the decerebrate cat the usual excitatory effect on the extensors of stimulating a nerve in the opposite leg was changed by chloroform into an inhibitory effect. This reversal appears before the effect of chloroform has gone far enough to abolish the reflex tonus in the extensor muscles which is necessary as a background for inhibition. Both the strychnine and chloroform reversals are rendered more intelligible by the concept of a twofold effect always present in response to any afferent stimulus. The conversion of a reflex effect into its exact opposite by a drug is a puzzling phenomenon until we recognize that two antagonistic elements in the central disturbance are always present; then the conversion of inhibition into excitation by strychnine appears as the abolition of the inhibitory content, enabling the excitatory content to come into evidence; and the conversion of excitation into inhibition by chloroform may similarly be interpreted as due to the abolition of the excitatory content of the disturbance.

OBSERVATIONS

The initial observation which struck me as being significant in connection with the above considerations and which led me to seek a repetition of it in other experiments, was as follows:

A cat was decerebrated under deep ether anesthesia with the decerebrating device described by Miller and Sherrington (11), the transection being about 3 mm. in front of the anterior edge of the anterior colliculi. Instead of a steady state of decerebrate rigidity the animal showed considerable spontaneous activity in the fore limbs, neck and trunk. In my experience, this is usually the result of a transection farther forward than that which gives typical decerebrate rigidity. Therefore, the animal was again etherized and a fresh transection made at the front edge of the anterior colliculi. The animal still showed much the same sort of activity as after the first transection, but was nevertheless pre-

pared for the demonstration of reciprocal innervation of antagonistic muscles. Stimulating electrodes were applied to the two sciatic nerves and all muscles of the right hind leg were paralyzed by nerve section except the vasto-crureus and the semitendinosus. The former was left with its normal attachments so that its contractions and relaxations should be shown by extension and flexion of the knee joint; the tendon of the semitendinosus was cut from its insertion and connected with a muscle lever to show separately the simultaneous behavior of a knee flexor. The reflexes were demonstrated, but the spread of reflex response to muscle groups in the anterior part of the animal was marked, and the almost constant activity of the fore limbs was distracting. Therefore the animal was again lightly etherized and a third transection was made through the middle of the anterior colliculi and close to the front edge of the posterior colliculi. After this transection the animal developed good typical decerebrate rigidity in the fore limbs, but little or none in the hind limbs. The sprawling activities disappeared, and the reflexes became more regular.

Shortly after this third transection, and while the animal was still under the effects of ether, it was noted that the crossed stimulus,—that is, stimulation of the left sciatic nerve,—even if strong, caused, instead of the usual full extension, only partial extension during stimulation, but a marked increase of extension when the stimulus ceased. This effect was observed repeatedly. As the ether wore off it became less pronounced. The effect appeared also to fatigue. If a second extensor stimulus was applied while the after-discharge of the first was still pronounced, the effect was not present. After a few minutes the animal was etherized again, and the effect became distinctly more pronounced, again becoming less so as the animal came out of ether. A third etherization produced the same result. No rebound contraction followed the inhibition of the extensors on stimulating the right sciatic nerve (flexion reflex), unless this reflex was evoked shortly after the cessation of the crossed stimulation; that is, during the time normally occupied by the after-discharge of the crossed extension reflex.

It was not feasible to prepare the necessary apparatus for making a permanent record from this animal, but five cats were subsequently decerebrated and prepared for recording this effect. In every case the transection was made in the neighborhood of the colliculi, usually about the middle of the anterior colliculi. In one case the transection was very close to the anterior edge of the cerebellum. In the first three experiments, including the one just described, the stimulating apparatus

consisted of a small inductorium such as is used for class work, operated by a buzzer interrupting the primary current approximately 50 times a second. The coil was not calibrated and no attempt was made to determine the strength of primary current. In the last three experiments a Berne coil was used which had been calibrated for break shocks in accordance with Martin's scale (12, p. 55). The primary current was measured with an ammeter and was interrupted by means of a rotary device so designed that the frequency of interruption and the duration of closure at each contact could be independently regulated at will.

The construction of this interrupter is as follows: On the shaft of a twelve volt D. C. motor is mounted a cylinder with six copper segments with which contact is made by two light copper brushes so designed as to have a natural period of vibration much more rapid than any frequency with which the apparatus is to be used. The six segments provide for six interruptions per revolution, and one of the brushes is so arranged that its position with respect to the other can be varied in such a way that the duration of closure each time the circuit is closed may be varied from 0 to 92 per cent of the time from the beginning of one closure to the beginning of the next. Coupled with this motor is a magneto connected with a voltmeter to serve as a speed indicator, and in the armature circuit is a carbon rheostat by which the speed may be regulated with considerable accuracy. It is possible to obtain frequencies of interruption from 25 to 270 per second. Tests with the string galvanometer when the apparatus was new showed perfectly maintained closure at all speeds tested. Recently a similar test has shown that at fairly high speeds one closure in each revolution, that is, one in six, is not perfectly maintained, and may thus result in the production of extra induction shocks. In these experiments the movable brush was so placed as to give 70 per cent of closure; that is, the break shock would occur after 70 per cent of the time had elapsed from one make shock to the next. This adjustment has been adopted as standard because Erlanger and Garrey (13) showed that the relatively great duration of make shocks may result in a reduction of the intensity of break shocks if the latter follow too close after them.

In the first four experiments the operative procedures were carried out exactly as described in the case of the first experiment. In the last two experiments no stimulating electrodes were applied to the right sciatic nerve, but it was cut at the hip, including the nerve to the hamstring muscles. All branches of the anterior crural nerve were cut

except those to the vasto-crureus muscle. The psoas muscles were cut, and as many branches of the obturator nerve as could be conveniently reached. In every experiment the femur was clamped vertically so that the foot would fall by gravity if the muscle relaxed. Thus all motions which could be recorded on the drum were excluded except those due to contraction or relaxation of the vasto-crureus muscle.

A strength of stimulus was selected at which a strong crossed extension reflex was obtained. A record of this was then obtained on a slowly moving drum, the time of application of the stimulus being recorded by a signal magnet. The drum was stopped and synchronous ordinates were obtained by again stimulatiug the nerve while the drum was stationary. Thus by measuring back from one of these synchronous ordinates it was possible to determine the point in the myogram corresponding with the cessation of the stimulus. After recording a few normal responses the animal was etherized and at intervals the response to similar procedure was obtained. The duration of stimulation was varied somewhat. In general it was maintained until the contraction of the muscle had practically ceased to increase. When the cessation of the stimulus was followed by the marked increase noted in the first animal, this would appear conspicuously on the record. As soon as the effect had appeared, or failing that, as soon as the crossed extension reflex had become reduced by the effect of ether to a very weak contraction, the ether was withdrawn and a series of records was taken as the effect of it was wearing off, until the reflex had returned to its normal character as unaffected by the anesthetic.

In every animal investigated the effect was found. In three out of the six it was marked; in the remaining three it was usually observable, but was not nearly so striking as in the other three. Figures 1 and 2 show typical records obtained from two of those animals in which the effect was marked. Figure 1 is taken from the animal in which the transection was made close to the cerebellum, and in this animal the effect was more marked than in any other. Ether had been given for 3 minutes from an ether bottle connected with a tracheal cannula. Four minutes after its withdrawal the first stimulus shown in the record was applied, the strength of stimulus being much greater than that required to produce a vigorous reflex before etherization. Just before this observation a somewhat weaker stimulus had failed to produce any reflex response. The second stimulus shown in the record was applied one minute after the first, the next two with intervals of $1\frac{1}{2}$ minutes preceding them, and the last two with intervals of 2 minutes preceding them.

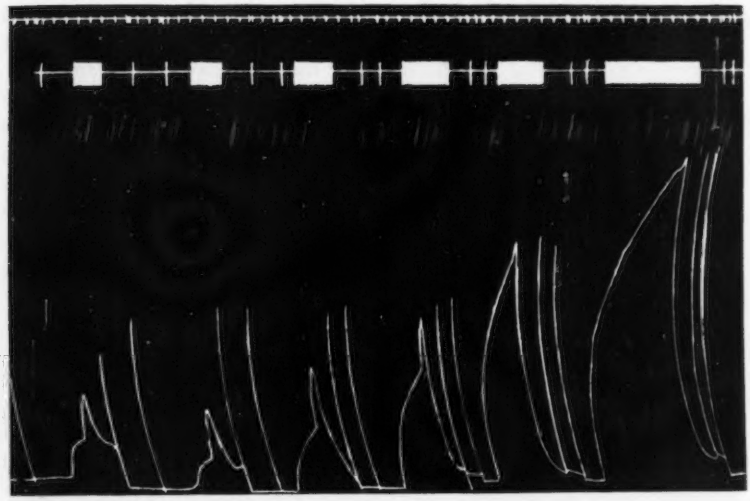


Fig. 1. Crossed extension reflex, vasto-crureus muscle; 4½ hours after decerebration (see text). In this and all subsequent figures including figure 6, ascent of the myograph line means contraction of the muscle. All read from left to right. Stimuli from uncalibrated coil. Time in seconds at the top.

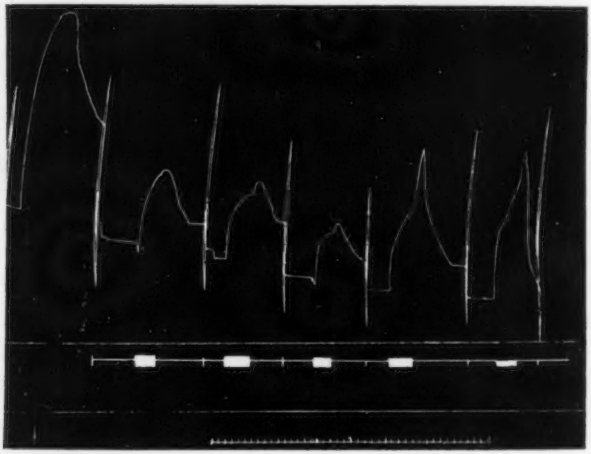


Fig. 2. Arrangement the same as figure 1; 2 hours after decerebration. Stimuli in first two observations, 88 Z units; in the remainder, 138. Primary current interrupted with rotary circuit-breaker 75 times a second. The speed of the drum was constant throughout. Time in seconds shown at bottom in part of the record.

Thus the last record, which shows an approximately normal crossed extension reflex with only a slight trace of the effect under discussion at the end, was obtained 12 minutes after the withdrawal of ether.

Figure 2 shows the effect in another of the preparations in which it was marked. The first contraction shown is the normal reflex, before ether was administered. Immediately after this observation ether was applied for 40 seconds. The second stimulus was applied a minute after the first. Between the second and third observations the strength of stimulus was increased, and the greater strength continued throughout the remainder. The last observation in the series was made 4 minutes after the first. Here a recovery from the ether has already begun to make the effect less pronounced.

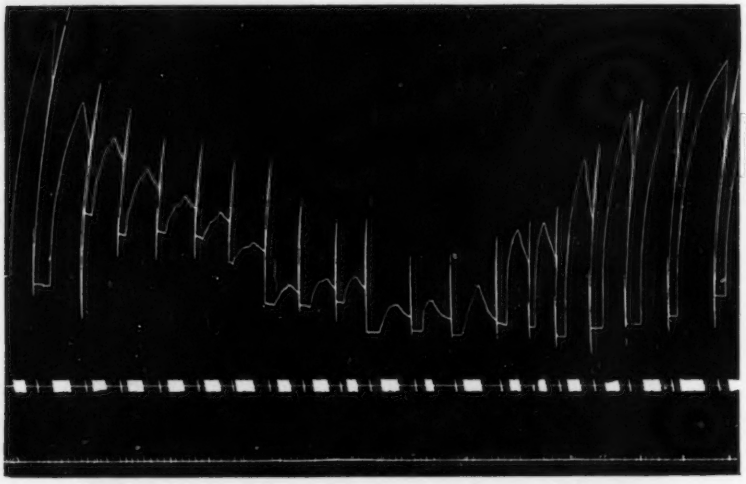


Fig. 3. Two and one-half hours after decerebration. Stimuli 170 Z units throughout. Interruption of primary current as in figure 2. Time in seconds at the bottom.

Figure 3 shows a typical series of observations from one of the animals in which the effect was least marked. This animal remained in good condition for a long time, and observations were made throughout the course of ten successive etherizations. The effect appeared to a slight extent, as is shown in the figure, during the onset of seven out of these ten etherizations. It failed to appear during the recovery from

ether. Ether was administered between the first and second observations shown in the figure, and withdrawn between the sixth and seventh observations. Fifteen minutes elapsed from the first to the last observation in the series and the intervals between the separate observations varied from 15 seconds to 2½ minutes. The strength of stimulus was maintained constant throughout the series.

DISCUSSION

General considerations. Graham Brown (14, fig 5, p. 348) has recorded a reflex response which shows superficially a close resemblance to the effect here described. This was obtained in a low spinal preparation 7½ hours after cutting the spinal cord. Contralateral stimulation produced extensor contraction, followed, on withdrawal of the stimulus, by a marked increase in the amplitude of contraction. In his observation there was no narcosis and the effect appears to have depended on a low spinal transection, a procedure which is well known to swing the "neural balance" away from extension toward flexion. He has also described a "narcosis progression" (15), (16) or rhythmic alternation between flexion and extension, occurring reciprocally in the two hind limbs under narcosis and independent of afferent stimulation. This may be related in some way to the effect here described, but the relation does not appear to be very close since the effect here described shows no evidence of rhythm, but merely an increase in extension whenever the stimulus ceases.

The partial abeyance, under the influence of ether, of the crossed extension reflex during the application of the stimulus which normally provokes it, appeared to me significant as illustrating an effect similar to the chloroform reversal of Sherrington and Sowton (10). Reflex excitation appears to have been in part converted into reflex inhibition. The release of reflex excitation on cessation of the external stimulus at once suggests the rebound contraction of extensors following their reflex inhibition in the majority of normal decerebrate preparations. The picture is most easily interpreted on the assumption that the external stimulus produces two antagonistic central influences—extensor and flexor; that is, with respect to the extensor motor neurones—excitatory and inhibitory (8). On this view the effect of ether is to narcotize the extensor element more than the flexor, and thereby prevent the usual dominance of the extensor effect during the application of the stimulus. But since extensor reflexes are notably characterized by a prolonged

after-discharge as compared with flexor reflexes (1, p. 30), the increased extensor activity following the withdrawal of the stimulus may be simply the expression of this difference in after-discharge. This view is in harmony with the interpretation of the reflex rebound proposed by Sherrington and Sowton already referred to in the introduction. Graham Brown has shown that the crossed extension reflex is abolished by general narcosis more readily than the flexion reflex (16, p. 30). This fact lends support to the view outlined above, for in the dual effect the extensor element normally prevails, and yet under ether it is visibly restrained by the flexor element as long as the stimulus lasts, and only becomes wholly dominant when the stimulus ceases.

The effect here described does not appear to have occurred in the experiments of Sherrington and Sowton in which they showed a conversion of reflex excitation into reflex inhibition by the action of chloroform (10). It is possible that this is due to a difference between the two drugs, but it seems to me more likely that the difference depends on the degree of narcosis or on the intensity or rhythm of stimulation. That the absence of this effect in their experiment may be due to the weakness of their stimulus as compared with mine, is rendered probable by the fact that their stimulus is expressly stated to have been weak, whereas in these experiments it was found necessary to use stimuli stronger than were required to produce a powerful crossed extension reflex before narcosis.

In their earlier paper they observed that merely changing the intensity of stimulation sufficed to cause a reversal of the central effect. Thus in their experiment with chloroform the stimuli may have been inadequate to excite the extensor mechanism in the central complex under the degree of narcosis then present, at least to the extent necessary to induce the requisite after-discharge. Sherrington (1, fig. 27, p. 77) has shown that the crossed extension reflex changes as the stimuli increase in strength chiefly by showing a much longer after-discharge; an observation which may be significant in this connection, since it is the after-discharge on which the effect depends.

Basis of analysis. The observations on which most of our knowledge of reflex behavior rests were made when the character of the nerve impulse, as revealed by the work of Lucas and Adrian (17), (18), (19), (20), (21), was unknown. It was customary to think of nerve impulses as streams of energy which could be graded in intensity by grading the strength of stimulus, much as one increases a stream of water by opening a faucet.

It is now well established that the nerve impulse obeys the "all-or-none" law (21); that the only gradations which can occur in a single response, aside from varying the number of conducting units excited, are due to the relative refractory phase (stage of recovery from previous response), unless there is a deranged state of the tissue through narcosis or other injurious condition. In any lapse of time beyond that required for a single response there is also the possibility of gradation in the frequency with which the responses follow each other in each conducting unit. There can hardly be a reasonable doubt that this law applies to all nerve fibers, afferent and efferent alike, since no morphological or functional differences have been found which indicate any divergence so profound as would be involved by an exception to this law.

It will be interesting to see how some of the phenomena of central antagonism, "neural balance," etc., can be interpreted in terms of this new conception of the nerve impulses of which the reflexes are built. In particular it will be of interest to see how far the explanation of reflex inhibition proposed by Lucas (17), (22) can be made to fit the facts of reciprocal innervation and the relationships observed between opposing central effects.

Gradation in reflex response strikingly follows gradation in intensity of afferent stimulus over a very wide range of intensities (23). Graham Brown (24), studying gradations in the reflex response of the tenuissimus muscle, concluded that each motor neurone must discharge with graded intensity. His stimuli were not single induction shocks, but a series of tetanizing stimuli; furthermore it has been shown (23, p. 205) that even a single induction shock of great strength may produce compound instead of simple stimulation. Thus the possibility of gradation in frequency is introduced and with it the possibility of central summation. His results therefore do not prove the existence of any other mode of gradation than those specified above. It has been shown in a previous paper (23, p. 211) that even after a single induction shock has been made strong enough to excite all the fibers of an afferent nerve, as judged by the fact that it provokes a maximal action current, further increments in the intensity of the induction shock can produce very great additional increments in the extent of reflex responses. The explanation of this seems to be compound stimulation. By this I mean that the stimulus provokes so profound a local disturbance that the local excitatory process persists through the refractory phase and is able to initiate a second and possibly a third propagated disturbance in a single nerve fiber. This must be borne in mind in appraising many

of the researches in which reflexes were studied under the effect of strong faradic stimulation.

We may start our analysis of reflex behavior with the assumption that all differences in afferent stimulation must depend ultimately on differences in the number of fibers stimulated and differences in frequency of impulses in the individual fibers, bearing in mind that in the case of strong stimuli the nerve impulses may have a higher frequency than the induction shocks which induce them, owing to the possibility of compound stimulation arising from individual shocks, and that at high frequencies the impulses will be of more or less subnormal (22) (and under certain circumstances (25), (26) supernormal) magnitude, according to the frequency.

Lutz (27), working on frogs, found that the average threshold of stimulation for the nerve-muscle preparation was about 4 Z units (Martin's scale) whereas the average threshold for the flexion reflex with electrodes similarly applied to an afferent nerve was about 8 Z units. By cooling the frog he raised the threshold for the nerve-muscle preparation by about 0.1 Z unit per degree C., whereas the threshold for reflex stimulation was raised nine times as much as this per degree C. (28). Since he worked with single induction shocks of threshold strength, and consequently almost certainly far too weak to produce compound stimulation in the nerve, each afferent fiber carried only a single impulse to the center; there was therefore no opportunity for summation of successive propagated disturbances. If individual reflex arcs were isolated so that each afferent fiber was connected with a single motor neurone and only one, then if such an arc were capable of transmitting a single impulse it should make no difference in the motor response of that arc how strong a stimulus was used, provided it was above threshold. The reflex preparation would then be analogous to the nerve-muscle preparation, but with one more junctional point in each conducting path. Under these conditions the difference in threshold which Lutz found between the nerve-muscle and the reflex preparations would depend on a difference in irritability between afferent and motor fibers. A difference as large as he found would on this assumption be surprising, but not impossible. But the great rise in the threshold of the reflex preparation on cooling as contrasted with that of the nerve-muscle preparation, would imply on any such basis a most improbable functional difference between afferent and motor fibers; it seems altogether unlikely that afferent fibers, morphologically so similar to motor fibers, should have nine times as large a temperature coefficient of threshold.

Accepting the all-or-none law and assuming isolated reflex paths, the only other possible explanation would be that cooling in every case established a central block in all the synapses except those which happened to be connected with those fibers in the afferent nerve which were relatively inexcitable or relatively inaccessible to the stimulating current. This involves a series of coincidences obviously most improbable. The most reasonable explanation seems to be that individual reflex arcs are not isolated, but that two or more afferent neurones converge at a single central neurone, internuncial or motor (cf. 28, p. 525, fig. 6). This does not necessarily imply that in a given collective reflex arc there are more afferent than motor fibers. It is conceivable that a group of ten afferent fibers might each make connection with every one of ten motor neurones. The extensive branching known histologically to exist in the grey matter would account for such interconnection to a high degree of complexity, and the large excess of afferent over motor neurones favors it. I have elsewhere shown (29) physiological evidence that individual motor neurones are accessible to afferent impulses from fibers in different nerve trunks which evoke the same reflex response. Impulses from two or more afferent fibers converging at synapses of a single motor neurone might suffice to set up in it an impulse or series of impulses which one approaching through a single afferent neurone would fail to do.

In making our analysis of reflex activity we may then take for granted the possibility of gradation in the frequency of impulses in each fiber and in the number of fibers involved, and furthermore the possibility of convergence of these fibers on individual central neurones.

Possible mechanism of reflex inhibition. The explanation of reflex inhibition proposed by Lucas (22), (17) is based on his analysis of the so-called Wedensky inhibition. Briefly stated, it is that in some portion of the conducting path involved in the reflex process, presumably the last internuncial neurone traversed by the disturbance before the motor neurone, the impulses follow each other with such frequency that each occurs during the relative refractory period following its predecessor, and consequently is subnormal; that the synapse through which the impulses must pass in order to reach the motor neurone constitutes a region of decrement through which these subnormal impulses cannot pass, although full-sized impulses such as would occur at a slower frequency of discharge could pass through. A neurone so occupied would establish an absolute block provided that only through it could the motor neurone be excited, for at all times it would be either absolutely

or relatively refractory and incapable of a larger response than those subnormal impulses already being conducted and extinguished at the synapse. In the subsequent discussion we may conveniently designate this the "pre-motor" neurone. *In such a neurone there would be correlated with the degree of decrement at the terminal synapse a critical frequency of nerve impulses above which the effect would be inhibitory, and below which, excitatory.*

The balancing of antagonistic central effects. In discussing this explanation Adrian (30, p. 45, cf. 17, p. 98) has mentioned as an objection Sherrington's observation (31) that if an excitatory and an inhibitory nerve are excited at the same time the effect produced is a simple algebraic summation of the two single effects (i.e., an intermediate degree of extension), and depends entirely on the relative strength of the two stimuli. It appears difficult to reconcile this fact with the proposed explanation, for (30, p. 45) "the frequency of the impulses in the central paths should be, if anything, greater when both nerves are stimulated than when the inhibitory nerve is acting by itself, and therefore the impulses should be still further reduced in intensity."

I have studied this same balancing of excitatory and inhibitory central effects in the vasto-crureus muscle of the decerebrate cat, using induction shocks from coils calibrated according to Martin's scale. In my experience stimulation of the afferent nerve on the same side as the extensor muscle observed, unless very weak, has always sufficed to produce complete inhibition, unless the central effect was complicated by a synaptic fatigue resulting from continued stimulation of that nerve (29, p. 112). Under similar experimental conditions Gregg and I found that induction shocks of 40 to 50 Z units were required to excite all fibers in the sciatic nerve of the cat as judged by the size of the resulting action current. It is desirable to know at what strength of inhibitory stimulus its effect can still be partly overcome by an excitatory stimulus.

I have examined a series of old records, some of which have been published (6), with a view to estimating the degree of inhibition, by measuring on the myograph the percentage of the total possible relaxation obtained by inhibitory stimuli of various strengths applied to the peroneal nerve. The relaxations have been measured in each case from the degree of contraction existing at the time the inhibitory stimulus was applied. In every experiment in the series the make shocks were subminimal, and the break shocks were delivered to each nerve at a rate between 40 and 50 per second. The results of this examination

are shown in table 1. For the sake of indicating when two or more observations were made on the same preparation, these are identified by the same numbers that were assigned to them in an earlier paper (6). These identification numbers are given in the first column. In the third column is mentioned the degree of decerebrate tonus existing at the time of the test, and to which was added the crossed extension reflex in all but three observations. In every case the knee was in approximately extreme extension when the inhibitory stimulus was applied. Preparation 5 was a high spinal preparation and therefore without

TABLE 1

PREPARATION	INHIBITORY STIMULUS, Z UNITS	TONUS	EXCITATORY STIMULUS, Z UNITS	PER CENT INHIBITION
3	17.0	Strong	No stimulus	65
3	18.0	Strong	No stimulus	89
3	17.0	Strong	45.0	22
3	18.0	Strong	45.0	30
3	24.0	Strong	72.0	95
3	26.0	Strong	72.0	35
3	26.0	Strong	72.0	78
3	26.0	Strong	72.0	45
3	26.0	Strong	72.0	30
3	26.0	Fair	72.0	78
3	26.0	Moderate	72.0	83
2	18.0	None	50.0	81
2	15.0	None	50.0	75
5	14.0	None	44.0	92
7	7.0	Slight	14.0	92
9	4.0	Medium	28.0	61
9	4.8	Good	No stimulus	82
10	5.8	None	6.4	95

tonus; all the others were decerebrate. The twelfth and eighteenth observations in the table are those appearing in figures 9 and 10 respectively in the previous paper (6). The decrease in inhibitory efficiency toward the end of the series in preparation 3 was probably due to some local impairment at the point of stimulation in the afferent nerve (cf. 23, fig. 1).

Reference to figure 1 (23) will show that since it required 40 Z units applied to the sciatic nerve to produce a maximal action current, and even though the lines of current are more concentrated in a smaller nerve such as the peroneal, the difference is not large, it is probable that none of the inhibitory stimuli shown in the table sufficed to excite

all the afferent fibers. Moreover in these experiments only the peroneal branch of the sciatic nerve was used, and it has been shown by Camis (32) that no one branch of the sciatic nerve can produce as strong a central flexion effect as can be produced by stimulation of the entire sciatic nerve.

These considerations seem to justify the way out of the difficulty which is proposed in the final chapter of Lucas's monograph (17, p. 99), namely, that a failure of complete inhibition in this center can only occur when a considerable number of afferent fibers remains unexcited and therefore a considerable proportion of the motor neurones remains free from the inhibitory effect.

An objection might be raised to the validity of these comparisons on the ground that Z units are not absolute but only relative units of intensity; that is, the stimulating value of the Z unit varies with the resistance in the secondary circuit. Under perfectly constant conditions, that is, with electrodes undisturbed in contact with a given tissue so that the secondary resistance cannot vary appreciably, Z units offer a fairly accurate standard of comparison between different successive stimuli, the error amounting only to about ± 2 per cent. In the comparison of stimuli applied to different types of tissue through different types of electrodes the variation in the stimulating efficiency is so great that the Z unit is of comparatively little value, but when stimulating electrodes of the same design and dimensions are applied in the same way to nerves of approximately the same size the conditions are so closely similar that the error in comparing the stimuli in successive experiments in Z units is probably not much greater than that in comparing successive stimuli in the same experiment. Porter (33) in a series of 52 determinations of the threshold of the nerve-muscle preparation in the spinal cat, each in a separate animal, with electrodes applied to the radial nerve, found only 21, or 40 per cent, differing from the average value by a ratio of more than two to one. Considering the possibility of real variation in irritability from animal to animal, and especially of local impairment of the nerve at the point stimulated due to handling, etc., this series may be considered good evidence of the approximate validity of the Z unit as a comparison for stimuli under similar conditions. The evidence shown in figure 1 of a previous paper (23) indicates that in appraising the real value of a stimulus far more error is likely to arise through local impairment of the nerve trunk, which usually occurs near the point at which the ligature is applied, than from differences in resistance in the secondary circuit or in the concentration of lines of

current flow when electrodes of the same design are applied in the same manner to either the peroneal or popliteal nerve in the cat (these nerves being approximately the same size).

It may probably be safely concluded that if with the electrodes applied as in these experiments maximal stimulation is only attained at about 40 Z units in the sciatic nerve, stimuli of 15 Z units or less similarly applied to either popliteal or peroneal nerve will almost certainly be submaximal; that is, will fail to stimulate all fibers. Thus we may reasonably dispose of the obstacle which Adrian recognizes as standing in the way of accepting the proposed explanation of central inhibition.

Electrical reversal. In Lucas's monograph significant facts are mentioned in support of this explanation of inhibition (17, p. 96). One is the reversal of reflex effect shown by Sherrington and Sowton to result from changing the character of electrical stimulation, already mentioned in the introduction; this may conveniently be designated "electrical reversal." The observed facts are that in the case of the extensor center faradization of the afferent nerve in the same limb, unless very weak, produces inhibition. Weak galvanic currents each lasting 0.04 second and succeeding each other at a rate of 12 per second, caused reflex excitation; currents of the same duration and frequency but approximately ten times as strong, caused reflex inhibition. Alternating currents of 20 cycles a second from a rheonome caused reflex excitation. Tiedermann is cited as showing that in a frog under strychnine, reflex excitation is converted into inhibition by an increase in the frequency of stimulation.

Lucas and Adrian (17, p. 96), (30) point out how the proposed explanation harmonizes with these facts, for in the so-called Wedensky inhibition which furnishes the model, an increase in frequency at the appropriate strength of stimulus, or an increase in strength at the appropriate frequency, would serve to convert excitation into inhibition. Further support is found in the fact that Tiedermann, and also Sherrington and Sowton, obtained with certain strengths and frequencies of faradization an initial twitch followed by inhibition (3, fig. 3), just as is found in the Wedensky inhibition.

Thus far the facts of electrical reversal fit the proposed explanation as well as could be desired. The implication is that when a series of stimuli applied to an afferent nerve causes excitation in the extensor motor neurones each impulse in an afferent fiber sets up a single impulse in the "pre-motor" neurone, and that when the frequency of afferent stimulation is increased by the required amount to produce cen-

tral inhibition, still each afferent impulse sets up a single impulse in the pre-motor neurone, but that now each comes nearly enough in the relative refractory period of this neurone to make the impulses subnormal by the amount required for their extinction at the final synapse. The application of the theory in this form to the observations of Sherrington and Sowton would require that the relative refractory period in the pre-motor neurone should last more than a 28th of a second, since inhibition is found with induction shocks of moderate intensity delivered at a frequency of 28 a second. The strengths of these shocks are not definitely given nor the primary current producing them, but from the coil distances it may be inferred that the shocks were probably not strong enough to produce compound stimulation. In my own observations strong inhibition was produced, as already stated, by induction shocks of only 10 Z units at frequencies between 40 and 50 per second. If the theory is to be applied without further complication it will require that the pre-motor neurone should still be only capable of conducting subnormal impulses, 0.02 second in my experiments, and 0.036 second in the experiment of Sherrington and Sowton, after the passage of a previous impulse. This would imply a refractory period far longer than is found in peripheral nerves in mammals at body temperature. However, this is not in itself necessarily a serious obstacle.

Greater difficulty is presented by the fact that Porter (33) has in a large number of spinal preparations evoked the flexion reflex by a single induction shock which varied in the different experiments from a minimum of less than 1 Z unit to a maximum of 21 Z units. These stimuli are presumably far too weak to produce compound stimulation in the afferent nerve. Sherrington has found that the flexion reflex regularly includes inhibition of the extensor muscles (1, p. 93), and if this can be evoked by a single induction shock too weak to produce compound stimulation, we cannot depend on any frequency of impulses in the afferent fibers for the causation of inhibition. If this is the case the application of the theory must become more complicated.

The question whether it is possible to produce reflex inhibition of a skeletal muscle by applying to an afferent nerve a single induction shock too weak to cause compound (rhythmic) stimulation, seemed to me crucial in deciding whether we may explain the phenomenon of electrical reversal in the direct and simple manner proposed by Lucas and Adrian. I have therefore made a series of experiments in a decerebrate cat to test the point. The transection was made at the anterior colliculi. The right leg was prepared for recording the contraction of the

vasto-crureus muscle as described earlier in this paper, the stimuli being applied to the popliteal nerve. After a series of records was obtained, the left gastrocnemius muscle was similarly studied, and finally the left vasto-crureus, the stimuli being applied in each case to the peroneal nerve. In the series with the left vasto-crureus the psoas muscle was not cut and only the saphenous and sartorius branches of the anterior crural nerve, since knee flexion must signify inhibition irrespective of the action of the hip flexors. The stimuli used were single break shocks, and the primary circuit was broken by the key described in an earlier paper, consisting of a sharp amalgamated copper point dipping into mercury. This key insures a clean break without risk of closure after the circuit is once broken.

In each of the three nerve centers examined in this animal, inhibition could be obtained with single induction shocks of moderate intensity. In the first test with the right vasto-crureus muscle the tonus was comparatively weak and the inhibitory effect did not regularly appear in a pure form. Sometimes it was complicated by an excitatory response and sometimes completely replaced by it. In the left gastrocnemius preparation inhibition was regularly obtained with single induction shocks varying from 5 to 80 Z units. In the left vasto-crureus center inhibition was regularly obtained with single shocks varying from 4 Z units to over 300. The inhibitory effect was rendered more marked by passive extension of the knee, before each test, the knee manifesting the "shortening reaction" (34); that is, remaining in a slightly more extended condition after passive extension than before. Figure 4 A shows a series of consecutive tests with increasing strength of induction shocks. Figure 4 B shows a similar series of tests taken immediately after those in figure 4 A with stimuli of progressively decreasing strength. In each case the drum was stopped, the knee passively extended, the drum then started, the stimulus applied, and after the reaction had been completed the drum was stopped again and the knee was again extended with the drum stationary, thus making between each two successive observations an ordinate on the drum. The entire series shown in figure 4 occupied somewhat less than 10 minutes; that is, the observations were made at a rate of between 2 and 3 a minute.

One of the afferent nerves used in this experiment was subsequently cut centrally and connected with a string galvanometer without disturbing the contacts of the stimulating electrodes. The galvanometer was of the Hindle type with a string of only 1.5μ diameter, and therefore adapted to responding rapidly to very small changes. Stimuli of

varying strengths were applied and the action currents were recorded monophasically. The responses became maximal when the strength of the break shocks had been raised to about 45 Z units; even with break shocks of more than 200 Z units no evidence could be found of a second action current in response to a single shock.

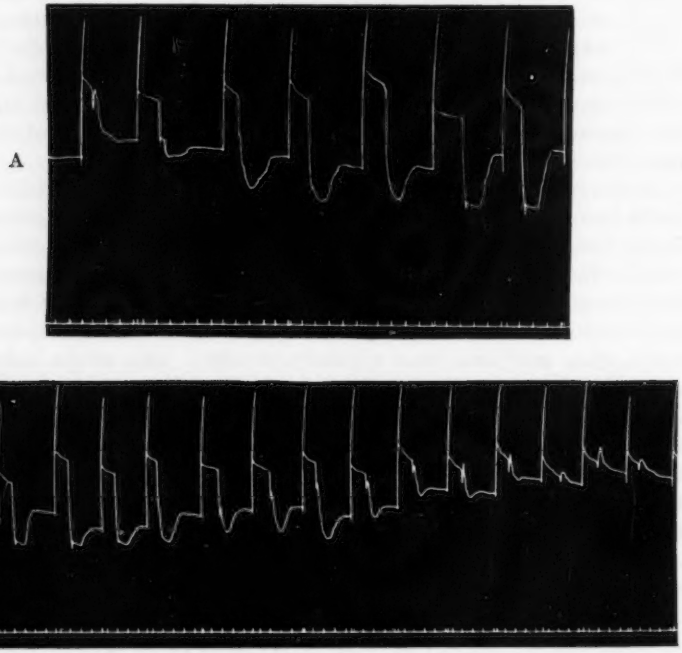


Fig. 4. Left vasto-crureus inhibited by single ascending break shocks applied to left peroneal nerve (see text). The stimuli taken in order were as follows: in A, 18 Z, 27 Z, 33 Z, 42 Z, 54 Z, 73 Z, 105 Z; in B, 149 Z, 105 Z, 73 Z, 54 Z, 42 Z, 33 Z, 27 Z, 22 Z, 18 Z, 13 Z, 10 Z, 8 Z, 6 Z, 5 Z, 4 Z. Speed of drum constant throughout. The records in A are photographed on a larger scale than in B. Time in seconds below.

Clearly inhibition can be caused by a single induction shock almost certainly too weak to set up more than one impulse in a single fiber; therefore the electrical reversal cannot be explained by the simple extension of the stimulation frequency into the pre-motor neurone. Inhibition must depend on something other than the peripheral stimu-

lation frequency. This does not mean that it cannot depend on a special frequency of impulses in the premotor neurone, as proposed by Lucas, but that if this frequency exists it must be set up in some other way. It may be that convergence of many afferent fibers at a single internuncial neurone will suffice to impart to it the necessary frequency of excitation. The conduction times of the different fibers converging on the synapse might differ enough to provide a rapid sequence of separate impulses arriving at the confluence of the dendrites. This explanation would imply a very high frequency, since the total reduced reflex time in the flexion reflex is about 4σ (35, p. 144), and it is unlikely that the conduction times of different afferent paths to the pre-motor neurone would differ from each other by more than a small fraction of this amount. It may be significant in this connection that Gregg and I found reason to infer that certain central portions of reflex conducting paths have briefer refractory periods than the afferent nerve fibers (23, p. 221).

It is quite conceivable that the change from inhibition to excitation on reduction of the strength of afferent stimulation might be explained on this same basis of converging afferent fibers. If the stimuli are reduced to submaximal strength fewer fibers would be stimulated, and consequently there might be fewer impulses set up by the different converging fibers in a given time; the frequency in the pre-motor neurone might thus be reduced below the critical value and thus become excitatory. Also the current of gradual onset from the rheonome, by initiating the individual impulses at different instants instead of simultaneously, might favor a lower frequency of arrival at the synapse on the part of the converging impulses. Thus through the principle of convergence we may arrive at a possible explanation of electrical reversal.

We must not forget that tonus has been found a prerequisite for this excitatory effect. Sherrington and Sowton found the ipselateral extensor contraction unobtainable in the decapitate preparation (3, p. 442), and in the decerebrate animal either absent or poor unless the reflex tonus of the muscle was good. This fact in itself shows that the excitatory effect cannot be conditioned solely by an appropriately low frequency of afferent stimuli; there must be a certain reinforcement through other central paths. How to fit such reinforcement into the scheme of critical frequencies in an internuncial neurone is difficult to see, although a possible method may be found with the aid of additional nervous connections for whose existence reasons will be shown presently.

Rebound. At this point we should consider the question of extensor rebound contraction following inhibition. An early view of this response was that the reflex energy of which tonus was the expression accumulates during inhibition, and upon its release breaks through with renewed intensity. Aside from the fact that in this way of looking at rebound we are treating tonus as if it were a stream of water, and ignoring the nature, mode of initiation and of propagation of the nerve impulses which make up reflex activity, the view is untenable for reasons which I have discussed elsewhere (6, p. 160). Sherrington found that the amount of rebound contraction following inhibition shows no correlation with the amount of tonic activity inhibited (4, p. 59); for instance, inhibition prolonged beyond a certain time is followed by less rebound than if the inhibition is briefer. Rebound therefore cannot depend merely on the accumulation of the suppressed tonus. Neither can it depend solely on the flexed position of the limb, for if so, the duration of inhibition (flexion) should make no difference; furthermore rebound occurs in the de-afferented preparation (14, p. 394), which would be impossible if it depended on proprioceptive impulses from the inhibited muscle. Apparently rebound depends on some central effect set up by the inhibitory stimulus itself, an effect which fatigues if this stimulus be continued long enough.

Looking at rebound from the point of view of nerve impulse frequency an attractive hypothesis for explaining it would be to suppose that during the afferent stimulation which produces inhibition the impulses converge at the pre-motor neurone with such frequency that the inhibitory rhythm is set up in it, but that on cessation of stimulation the frequency of impulses impinging on this neurone decreases till the rhythm in it falls below the critical value and becomes excitatory. This is quite conceivably so, but it requires some further assumption to account for the persistence of a series of impulses impinging on the pre-motor neurone for several seconds after the stimulation of the afferent nerve has ceased. Some central mechanism must be excited which continues to transmit impulses to the motor neurones for a long time after the afferent impulses have ceased to come in. We may possibly account for this fact without postulating in the central complex any properties foreign to peripheral conducting paths, if we assume a sufficiently extensive series of branching paths in which conduction is delayed, as it probably is in the synapses. A single impulse might, through extensive branching of the fiber which conducts it, set up other impulses in a large number of central neurones, and these in turn might

set up impulses in other neurones, and the synaptic delays in some of the more extensive paths might suffice to account for the observed continuance of the motor response through many seconds; and yet there need not exist in each conducting path any functional property which has not been demonstrated in the nerve-muscle preparation.

The same considerations apply to the crossed extension reflex, in which the after-discharge is perhaps its most striking feature. Sherrington has shown a crossed extension reflex with an after-discharge of more than 12 seconds (1, p. 77). This is probably in part due to the secondary reflex effect originating in the proprioceptive impulses coming from the extensor muscle itself. Sherrington has shown that the "shortening reaction" is attributable to these afferent impulses (34), and the after-discharge of the crossed extension reflex is undoubtedly to some extent a kindred phenomenon. On the other hand a considerable after-discharge can occur when all these proprioceptive impulses are completely cut off, as is shown by the after-discharge amounting to several seconds occurring in the crossed extension reflex in the de-afferent preparation (6, fig. 16).

Additional instances of delayed reflex response which may be likened to the after-discharge in the case of the crossed extension reflex are shown in figure 4 B and figure 5, the latter being obtained from the right vasto-crureus preparation in the same animal whose left vasto-crureus furnished the records in figure 4. In each case induction shocks of moderate intensity, and therefore almost certainly setting up only a synchronous volley of single impulses in the afferent nerve, provoked complex central responses involving alternations between flexion and extension; i.e., between excitation and inhibition (cf. 14, p. 372). They illustrate the initiation of complex central effects far outlasting the arrival of the afferent impulses in the central structures, and therefore demanding for their execution far more than simple unbranched conduction to the pre-motor neurone. In this connection we should recall the observation of Dreyer and Sherrington (36) which led them to conclude that with single induction shocks of moderate intensity, "repetitive volleys" of impulses are evoked in the motor neurones involved in the simple flexion reflex. The triphasic responses appearing in figures 5 and 6, beginning with excitation, changing into inhibition, and ending with excitation, might be explained if we suppose the initial contraction to be due to the first full-sized impulse set up in each pre-motor neurone before the inhibitory frequency has been established and the final contraction to be caused by a decrease in frequency such as was suggested to explain rebound.

From the various facts enumerated above we must conclude that to accept the proposed explanation of inhibition we must recognize that the frequency of impulses in the pre-motor neurone does not correspond

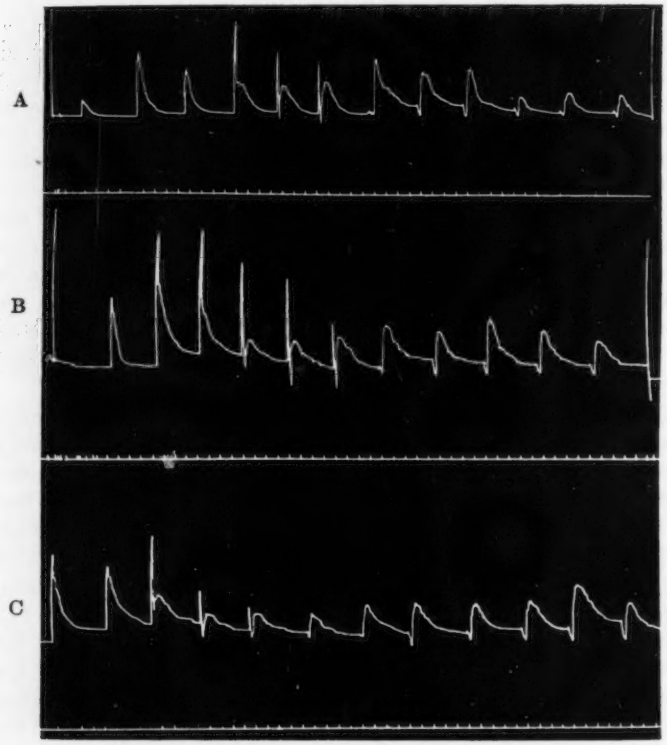


Fig. 5. Right vasto-crureus muscle. Single ascending break-shocks applied to right popliteal nerve. In each series the drum was not stopped between observations. The secondary coil distance was decreased after each stimulus. Stimuli in Z units as follows: A and C, 46, 59, 81, 116, 164, 240, 319, 408, 484, 545, 593, 631; B, 36, 46, 59, 81, 116, 164, 240, 319, 408, 484, 545. Time in seconds below.

directly with the frequency of impulses in the individual afferent fibers, and may indeed be only very indirectly dependent on that frequency (a condition which may be fulfilled by convergence of afferent fibers at a common central neurone); and furthermore, afferent impulses must in

some cases excite in the center some mechanism which enables the motor response to outlast the influx of afferent impulses far more than could be explained simply by their direct convergence upon the pre-motor neurone. There must be more elaborate paths than the simple termination of afferent fibers at the pre-motor neurone to account for the various delayed effects, but such paths need not preclude the presence of direct connection as well.

Chloroform and ether reversal. We must now consider the conversion of reflex excitation into reflex inhibition under the influence of chloroform, described by Sherrington and Sowton, and with it the transitional phenomenon under the influence of light ether anesthesia which forms the primary subject of this paper. In the final chapter of Lucas's monograph it has been suggested that this effect of chloroform may be readily explained by its known action in producing in a peripheral nerve a decrement in conduction. It is well known that nerve centers are more sensitive to the action of chloroform than nerve trunks, and therefore it is quite reasonable to suppose that if the synapse is a region of decrement, then at an early stage in the action of chloroform this decrement would be increased to the point of extinguishing those impulses which were previously able to pass the final synapse. Impulses traversing the pre-motor neurone at a frequency such that, in absence of chloroform, each was large enough to traverse the final synapse and excite the motor neurone, might fail to do so when the decrement at this final synapse had been increased by the action of the drug. In this way excitation might be replaced by inhibition.

Next comes the question whether this explanation will cover the facts shown in this paper; namely, the partial suppression under ether of the crossed extension reflex during the application of the stimulus, followed by a marked increase in extensor contraction as soon as stimulation ceases. There is no reason to suppose that ether will increase the decrement in every synapse in a spinal center by precisely the same amount. Let us suppose, therefore, that in some of the motor neurones the decrement is increased enough to make the existing frequency of nerve impulses in the pre-motor neurone inhibitory, but that in other motor neurones the effect has not sufficed to make this change. Under this condition we shall get during the application of the stimuli inhibition in some neurones and excitation in others, and consequently the observed intermediate degree of contraction in the muscle.

But this explanation alone will not account for the increased contraction following cessation of the stimuli. We have still to explain

the fact that some of those motor neurones which are inhibited during afferent stimulation are excited when it ceases. The crossed extension reflex is normally characterized by a prolonged after-discharge, and we have already found it necessary to postulate some elaborate conducting paths involved in this reflex arc. In this way we have established a condition for the continuance of motor response after afferent stimulation has ceased, but we have yet to find a mechanism for the sudden replacement of inhibition by excitation. It is possible that we may invoke the explanation proposed for rebound contraction, namely, that the impulses converging at the individual pre-motor neurone during afferent stimulation arrive with an inhibitory frequency, but that after stimulation has ceased they arrive less and less frequently. Some arcs with an increased decrement at the final synapse would then become inhibitory while impulses were crowding in rapidly as a result of afferent stimulation, but would still have a small enough decrement for the impulses at the reduced frequency (after afferent stimulation has ceased) to pass through. This view simply applies the principle of convergence already proposed to explain rebound in the homolateral arc, to the terminal effect in the more complex arc involved in the crossed extension reflex with its characteristically long delay. But it requires that the impulses from the crossed arc shall arrive at a given pre-motor neurone with a lower frequency than in the case of the homolateral arc, since in absence of narcosis the normal response even to the most powerful afferent stimulation is excitation instead of inhibition.

Just as the narcotic reversal may be explained on the basis of increased decrement in the final synapse, so the opposite reversal by strychnine (9), (37) (conversion of inhibition into excitation) might be explained on the basis of diminution of decrement. If the decrement in these synapses largely disappears, a frequency of impulses in the pre-motor neurone, normally inhibitory, would become excitatory.

It should be mentioned that Bayliss (38) has found effects of chloroform and strychnine on the vasomotor reflexes quite similar to those which Sherrington and his co-workers have found in the case of the limb reflexes, and it is probable that the ultimate explanation of one group of phenomena will hold good, at least in part, for the other.

Reciprocal innervation. Thus far the discussion has dealt wholly with the extensor motor neurones. In any working scheme we must allow for the reciprocal effects in the flexor motor neurones. The same stimulus which inhibits the extensors regularly excites the flexors (1, p. 93); the afferent fibers through which we excite the flexor motor neu-

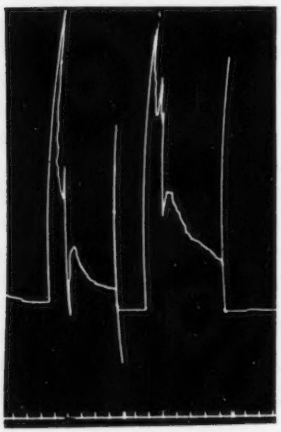
rones presumably give off branches which inhibit the extensors. This mechanism may be harmonized with the proposed scheme by assuming that the convergence of paths at individual pre-motor neurones in the flexor center is such that a lower frequency results in them from the simultaneous excitation of the afferent fibers than in the extensor center. Or the same result may be obtained if the decrement in the final synapse in the flexion reflex arc is less than in the corresponding synapse of the extensor arc, so that a frequency which in the pre-motor neurones of the extensor center is inhibitory, will in those of the flexor center be below the critical frequency, and therefore excitatory. That even the simple flexion reflex, evoked by a single induction shock, involves more than one impulse in each motor neurone, is suggested by the observations of Dreyer and Sherrington (36), although Gregg and I (35) were unable to find evidence to substantiate this view, except in the case of induction shocks so strong as to produce compound stimulation (23).[1] If the reflex involves only a single impulse in each pre-motor neurone, the question of frequency does not arise, since this neurone, if not already occupied, will then conduct a full-sized impulse which will readily pass through the final synapse and excite the motor neurone.

The inhibition of flexion during the crossed extension reflex could conceivably depend on convergence of fibers at the individual pre-motor neurone in the flexor center, imparting to it an inhibitory frequency of impulses. This presents a difficulty due to the fact that maximal afferent stimulation produces a flexion reflex which cannot be inhibited by any contralateral stimulus. Reflex inhibition which in the extensor center under maximal ipselateral stimulation constitutes an absolute block, fails in the case of contralateral flexors to block the reciprocal excitatory effects of strong ipselateral stimulation. According to the proposed explanation by frequency, a block should be absolute; no amount of stimulation of other nerves should break through it. In a later section we shall find reason to assume additional connections which will provide a different explanation of the inhibition of contralateral flexors, and thus offer a way out of the difficulty.

Postural reversal. We now come to the postural reversal or "Umkehr," which has been described by Sherrington (39, p. 299) and also studied by Magnus (40). In general this means the determination of the type of response to a given stimulus by the limb posture existing when the stimulus is applied. In the old-fashioned terminology this

[1] cf. Sassa and Sherrington: Proc. Roy. Soc., 1921, xcii, 108.

is described as a "tilting of the neural balance." Sherrington has found that certain stimuli will cause flexion if the limb is already passively extended, and extension if it is flexed. Magnus has found that the cat's tail when stimulated at the tip is reflexly drawn toward the median plane from whichever side it happens to be hanging. He has shown that this phenomenon depends upon proprioceptive impulses; that is, afferent impulses coming from receptors within the muscle. Since passive flexion stretches the extensor muscles and favors reflex extension, it is supposed that the afferent impulses from a stretched muscle predispose to an excitatory response the motor neurones of that muscle, and this view is supported by the above-mentioned observation of Magnus. Graham Brown (41) has found in the guinea pig a reversal in the opposite sense, due to actively maintained posture; when the limb was actively in a state of flexion the reflex response was further flexion, and when it was actively extended the reflex response was further extension. The two facts might be reconciled as both due to increased state of tension within the muscle, since either passive flexion or active extension will increase the tension in the extensor muscle. On the other hand I have obtained evidence of postural reversal in the same sense as that shown by Sherrington and Magnus even in the case of actively maintained posture. This is shown in the comparison between figures 5 and 6. Figure 6 was from the same animal, and made after the observations shown in figure 5 B and immediately before those in figure 5 C. In figure 5 each stimulus was applied after the limb had returned to a semi-flexed posture following the preceding stimulus. In figure 6 two observations are shown, in each of which the crossed extension reflex was evoked by pinching the toe of the opposite foot; during the subsidence of this response and while the limb was still considerably extended, a single shock was applied (as in fig. 5) to the ipselateral nerve. In figure 6 A the stimulus was of the same strength as in the third observation of figure 5 C. In figure 6 B the stimulus was the



A B

Fig. 6. Right vasto-crureus muscle (see text). Stimuli, single break shocks: A, 81 Z; B, 59 Z.

same as in the second observation of figure 5 C; it was applied just after the second peak in the myogram, the response to the stimulus of pinching being in itself rhythmic. In every one of the four observations under comparison the response is triphasic, first excitatory, then inhibitory, then excitatory. In each case when the stimulus was sent in during the state of reflex extension the inhibitory part of the response was very much increased in magnitude. This fact argues against harmonizing the reversal of Graham Brown with that of Magnus and Sherrington on the common ground of tension, for presumably the tension is greater during this state of active contraction than in the less active state, although it may be less in certain idle fibers, and it is conceivable that these idle fibers form a majority of the whole muscle and that their lack of tension is what determines the character of the response. In the experiment shown in figure 6 the motor nerves to the flexor muscles were cut, and therefore any proprioceptive afferent impulses influencing the result must have come from the extensor muscle itself.

In opposition to the view that the tension irrespective of the degree of shortening is what determines the nature of the afferent effect on the central response, may be cited the observations of Sherrington on "plastic tonus" (34), (42). He described what he calls the "shortening reaction" and the "lengthening reaction" in the vasto-crureus preparation of the decerebrate cat with moderate degrees of decerebrate rigidity. In the "shortening reaction" when the extensor muscle is allowed to shorten by passive extension of the knee it "takes up slack" and remains shortened; in the "lengthening reaction" when the knee is forcibly flexed there is inhibition of decerebrate tonus in the muscle and the knee remains flexed. Sherrington showed that these responses are reflex in nature, depending on afferent impulses arising in the vasto-crureus muscle itself. He further showed that in the case of the "shortening reaction" essentially the same thing happened if the muscle was reflexly contracted, and that in either case it was the shortening of the muscle—it made no difference whether actively or passively produced —that provided the adequate stimulus for the central effect. In short, the degree of shortening, irrespective of tension, seems to be the condition that determines what afferent impulses are set up. This fact suggests the view that the predisposing influence on the central response which Magnus and Sherrington have found traceable to posture, probably depends on the effect of actual shortening or lengthening of the muscle rather than on the amount of tension. This view would har-

monize the observations shown in figure 6 with the observations of Sherrington and Magnus.

The reversal in the guinea pig described by Graham Brown seems to be at variance with this principle. It should be recalled that this reversal was most marked in the intact animal in which the cerebral cortex was free to modify reflex behavior, and that it was only with difficulty obtained in the decerebrate animal. Moreover, the stimulus used to evoke the reflexes, manipulation of the "femoral fold" of the skin, was not very well adapted to qualitative regulation or to accurate limitation of the stimulus to the same fibers in every test. The apparent contradiction may be attributable to these considerations, or it may be due to a difference in the organization of the guinea pig's centers from those of the cat, with respect to postural regulation.

The explanation of the divergence, proposed by Graham Brown (41, p. 287), does not harmonize well with the knowledge of the nerve impulse which has come to light since his paper was published. He suggests that the actively maintained posture is an expression of a heightened state of excitation in the center of the tonically contracted muscle, and that to this excitation is added that induced by the sensory stimulus. We now know that a nerve impulse cannot be increased in magnitude by any increase in the stimulus which initiates it. On the other hand, such an effect as he invokes might under certain conditions be simulated by a summation of propagated disturbances such as Adrian and Lucas have described (25).

In fitting these facts of reversal into our proposed scheme we must remember that in general increased convergence of impulses at a pre-motor neurone decreases the chance of excitation of the motor neurones and increases the chance of inhibition. Let us assume that shortening of the extensor muscle, active or passive, sets up afferent impulses of a certain frequency, and that these set up impulses in the pre-motor neurones of the extensor center; we have then the condition for the "shortening reaction." Following our proposed scheme, the resulting frequency in the pre-motor neurones must be too low to be inhibitory. Those afferent stimuli whose central effect is subject to postural reversal may in themselves deliver to the pre-motor neurones in the extensor center impulses of a frequency also below the critical value. If when this stimulus is applied the limb is in a flexed posture and no impulses are coming to the pre-motor neurones through the afferent fibers from the extensor muscle itself, then the stimulus will cause an excitatory response, resulting in extension. But if the limb is already extended,

with the result that impulses of an excitatory frequency are already being set up in the pre-motor neurones by the afferent impulses from the muscle, and then if the external stimulus causes additional impulses to impinge on these pre-motor neurones, we shall have the condition for inhibition, because their effect will be to increase the frequency of impulses in these neurones above the critical value. Flexion will therefore result. In this way the proposed scheme might provide a mechanism for the "Umkehr" or postural reversal of reflex effect.

Before leaving the subject of postural effects we should note that the histologists have found two distinct types of sensory nerve endings in skeletal muscles,—free nerve endings and those which terminate in the structure of the muscle spindle (43). By assigning different functions to these two types we might find a further means of explaining inharmonious observations, but we should be adding to the complexity of a picture which has already been complicated beyond the indications of experimental fact.

Intrinsic alternation of flexion and extension. One more phenomenon of fundamental importance in the behavior of nerve centers remains to be considered,—the tendency to rhythmic alternation between flexion and extension even after all afferent impulses have been eliminated. Sherrington (44) has shown that the scratch reflex proceeds with unaltered rhythm in response to appropriate cutaneous stimulation, even after severance of all afferent fibers coming from the muscles involved. More recently Graham Brown (16) has studied the rhythmic alternation between flexion and extension which is characteristic of progression. This rhythm is slower than that of the scratch reflex, being usually one to two beats a second. He found that the intact animal under general narcosis tends to exhibit these movements spontaneously. At a moderate depth of narcosis transection of the spinal cord in the lumbar region will interrupt them. Their independence of afferent impulses is shown by their persistence after the severance of all afferent fibers from the limb under observation, and especially by their persistence at a depth of narcosis at which neither strong stimulation of a nerve in the opposite leg nor transection of the spinal cord produces any response. The progression rhythm persists under increasing narcosis almost if not quite as long as respiration. The rebound contraction disappears much earlier in narcosis than the progression rhythm.

Graham Brown proposes as an explanation (16, p. 37) that the motor neurone sends off a branch-fiber through which it may inhibit activity in the motor neurones of the antagonistic muscle. He mentions mor-

phological evidence of a branch leaving the axone of the spinal motor neurone not far from the cell body. To account for the rhythmic alternation he suggests that this inhibitory effect may become fatigued and thus in time enable the inhibited neurone to become active and in turn inhibit its antagonist.

Let us see how this phenomenon can be treated in accordance with our scheme of analysis. If we assume that the collateral branch of the motor neurone leads to the pre-motor neurone of the antagonistic center

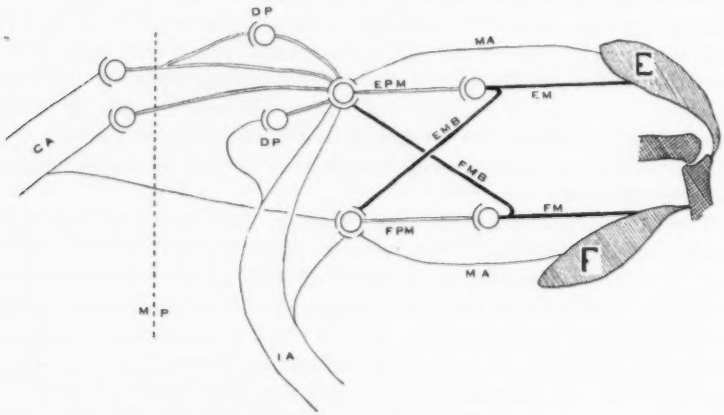


Fig. 7. Diagram of spinal neurones and their connections to provide a possible basis for analysis of reflexes. Afferent neurones shown in light lines; internuncial neurones in double lines; motor neurones in heavy lines. *E*, extensor muscle; *F*, flexor muscle; *M P*, median plane; *C A*, contralateral afferent fibers; *I A*, ipselateral afferent fibers; *D P*, delay paths, representing extensive central connections to provide for prolonged after-discharge; *E P M*, extensor pre-motor neurone; *F P M*, flexor pre-motor neurone; *E M*, extensor motor neurone; *F M*, flexor motor neurone; *E M B*, collateral branch of extensor motor neurone; *F M B*, collateral branch of flexor motor neurone.

(fig. 7) we shall at once establish the condition for converting excitation into inhibition in that center. Thus if a pre-motor neurone in the extensor center is at a given moment conducting impulses with a frequency below the critical value, and therefore causing excitation in the motor neurone, then if a series of impulses is set up in the flexor motor neurone, these will by virtue of the collateral branch leading to the extensor pre-motor neurone, set up in it additional impulses which will raise the frequency therein above the critical value, and inhibition will

result. The same picture may be reciprocally applied to the inhibition of flexors through activity in the extensor motor neurones. But the question at once arises, if impulses in the extensor motor neurone suffice to set up in the flexor pre-motor neurone impulses which combined with those from another source will be of inhibitory frequency, how can a flexion reflex be produced by afferent stimulation? In point of fact, we know that moderate stimulation of an appropriate nerve will inhibit the strongest extensor contraction and produce a flexion reflex. This may be explained as the result of the direct convergence of the afferent fibers at the extensor pre-motor neurone producing in it an inhibitory frequency in the manner already discussed. The resulting inhibition of the extensor motor neurone would stop the stream of impulses in the branch leading to the flexor pre-motor neurone and leave the latter free to be effectively excited through the afferent path. We may also invoke the aid of synaptic fatigue, a condition closely paralleled in the nerve-muscle junction and actually demonstrated in certain reflexes (29). The synapse at which the collateral branch of the extensor motor neurone makes contact with the flexor pre-motor neurone may fatigue rapidly and become non-conducting. If this were so then the inhibitory effect on the flexor center of impulses in the branch of the extensor motor neurone would soon wear off and the flexor pre-motor neurone would be free to respond to an excitatory stimulus without being first released in the roundabout way outlined above. Such a synaptic fatigue seems necessary to provide for the intrinsic progression rhythm described by Graham Brown as occurring in absence of rhythmic afferent stimulation.

The connections described will with the aid of synaptic fatigue explain the rhythmic alternation between flexion and extension, provided there is some initial source of nerve impulses in the central (pre-motor) neurones. Indeed some such source must exist no matter how we organize the neurones or what connections we provide. The scheme outlined provides for the intermittent interruption of impulses first in one set of motor neurones and then in the other, but what is to start them again? Either we must assume something in the grey matter of the spinal cord which can initiate nerve impulses, something possibly excitable by a so-called blood stimulus, or we must assume that through some such elaboration of branching paths as was suggested to explain after-discharge, a small disturbance, once started, is kept alive and enabled to excite a neurone again after the stage of inhibition is past.

It is possible that we might greatly simplify the entire scheme by relegating most of the inhibitory effects we have examined to the collateral branches of the motor neurones which Graham Brown has postulated for the explanation of progression rhythm. If we could explain the reciprocal innervation of antagonistic muscles as due to the effect of these branches in raising the impulse frequency in the antagonistic pre-motor neurone above the critical value, and thus make inhibition of one center secondary to the excitation of motor neurones in the opposite center, we should avoid the need of the somewhat difficult assumption made at the start, that the convergence at the individual pre-motor neurones of afferent fibers with different conducting times is responsible for imparting to them the inhibitory frequency. By invoking the aid of these branches we should make the inhibitory frequency depend on the arrival of impulses from them at the pre-motor neurones in alternation with those coming directly through the afferent fibers. In particular, this explanation would clear away the difficulty we met in trying to explain crossed (contralateral) inhibition of flexors by convergence of afferent fibers, for if inhibition of the flexors depends on joint action through the opposite afferent nerve (fig. 7, *C A*) and the collateral branch, *E M B*, of the extensor motor neurone, then all that is necessary to release the flexor motor neurones from inhibition is to stop the discharge of impulses in the extensor motor neurones. But here we must introduce a difference between flexors and extensors to account for the dominance of the flexion reflex. It must be possible to inhibit the extensor center without first initiating impulses in the flexor motor neurones; otherwise it would be impossible to break through the block of inhibition in the flexor center, as is done by a powerful ipselateral stimulus. To this end we must retain the idea of inhibition of extensors by convergence of afferent fibers, *I A*; but the crossed inhibition of flexors may well depend on the combined frequency of impulses arriving through afferent fibers and through collateral branches of extensor motor neurones.

There is another argument for this differentiation between the mechanisms of flexor and extensor inhibition. We have seen that the progression rhythm in absence of afferent impulses requires rapid fatigue in the synapse at which the collateral branch approaches the opposed premotor neurone. But if inhibition always depended on these branches and not on direct afferent convergence as at first proposed, then how could we reconcile the assumed rapid synaptic fatigue with the observed fact that strong afferent stimulation will keep decerebrate rigidity in

the extensors inhibited for a long time? This might be explained if the synaptic fatigue did not readily progress beyond a certain point at which it established a decrement sufficient to block very small impulses such as would occur if they followed each other with high frequency, but insufficient to block the larger impulses occurring at lower frequencies. The persistently dominant flexion reflex would on this view involve the slower frequency of impulses. In this connection it should be noted that in earlier experiments on an inhibitory reflex I have observed fatigue involving only the particular reflex arc employed, and probably therefore occurring in the synapse (29, p. 116). But such an explanation becomes unnecessary if we adhere to the view of afferent convergence to account for inhibition of extensors.

It will be recalled that the reinforcement by tonus of the excitatory phase of the electrical reversal, described by Sherrington and Sowton, appeared difficult to reconcile with the explanation of inhibition by the convergence of afferent fibers. This difficulty can perhaps be met with the aid of "summation of propagated disturbances," (25) enabling an afferent impulse appropriately timed after other impulses from the hind-brain to get through a synapse where the decrement is too great for a single impulse to pass.

We might attempt further analysis by assuming that the collateral branches of the flexor motor neurones are less subject to synaptic fatigue than the extensors, or that few reflexes involve all the motor neurones of a center at once, they being usually active and idle in rotation, or by assuming that in many cases when a chain of connections is traced from the flexor center through the collateral branch to the extensor center and back to the flexor center, it does not lead back to the same individual neurone from which it started, or by introducing the additional assumption that two or more pre-motor neurones may converge at a single motor neurone and thus provide a higher frequency in the collateral branch than that in any one pre-motor neurone contributing to it. But all these maneuvers are so complex and so speculative that it seems hardly worth while to follow them.

CONCLUSION

This analysis of reflex organization has led into many details which have little or no foundation either in experiment or anatomy, and which may therefore seem far-fetched and improbable. It would be simpler and perhaps more satisfactory to recognize merely two antagonistic

central effects induced by every afferent stimulus, as was done in the introduction, and to assume that these effects depend merely on the excitation of the two antagonistic mechanisms or groups of neurones each so arranged as to produce its appropriate effect on the motor neurones. Such a simple assumption would not conflict with the explanation of inhibition proposed by Lucas, for we might assume that each group of neurones in some way has the property of imparting to the pre-motor neurones of one center a relatively slow and therefore excitatory frequency of nerve impulses, and to the pre-motor neurone of the opposite center a more rapid and therefore inhibitory frequency; but such a simple assumption would fail to explain how these groups of neurones contrived to impart these determining frequencies to the pre-motor neurones. We should be leaving the analysis incomplete and describing the effect without attempting to explain the method. The more elaborate analysis as proposed furnishes a complete set of possible if not probable details for explaining all the facts considered without invoking any peculiar principle of reflex behavior or any functional capacity which has not been clearly demonstrated in the simple conducting paths constituting the nerve-muscle preparation, which have been so thoroughly analyzed in the laboratory. The proposed analysis is not set up as being in its present form at all probable. The underlying idea in carrying it as far as I have has been that expressed by Keith Lucas (17, p. 2) as follows: "Are we to suppose that the central nervous system uses some process different from that which is the basis of conduction in peripheral nerves, or is it more probable that the apparent differences rest only on our ignorance of the elementary facts of the conduction process? If we had a fuller knowledge of conduction as it occurs in peripheral nerve, should we not see Inhibition, Summation, and After-discharge as the natural and inevitable consequences of that one conduction process working under conditions of varying complexity?" As a method of attacking the problem he suggests—"that we should first inquire with all care whether the elementary phenomena of conduction, as they are to be seen in the simple motor nerve and muscle, can give a satisfactory basis for the understanding of central phenomena; if they cannot, and in that case only, we shall be forced to postulate some new process peculiar to the central nervous system." Guided by this idea I have sought to examine those of the more definitely established facts of reflex behavior with which I am acquainted, with a view to seeing if the known functional capacities of the peripheral conducting tissues could be made a possible basis for explaining them all.

The result of the analysis is of course inconclusive in the present state of our knowledge; it neither proves nor disproves the applicability of Lucas's proposed explanation of inhibition to reflex phenomena. But I believe it is worth recording that the attempt has as yet brought to light no insuperable obstacles.

SUMMARY

1. The decerebrate cat under a certain degree of ether narcosis exhibits a modification of the crossed extension reflex, as follows: During the application of a strong tetanizing stimulus to an afferent nerve in the hind limb, the knee extensor muscle in the opposite hind limb exhibits instead of the usual full contraction, a partial contraction during the application of the stimulus, followed on its cessation by a distinct increase in contraction. In some preparations this increase is marked, in others only barely discernible. The effect disappears as the animal is allowed to come out of ether.

2. This effect is similar in one respect to the conversion of reflex excitation into inhibition under chloroform, described by Sherrington and Sowton; in another respect it is similar to the rebound contraction occurring in extensor muscles after reflex inhibition. Taken in connection with these observations and with the strychnine reversal of reflex effect and the electrical reversal described by Sherrington and Sowton, this observation tends to support the view that in general the stimulation of a single afferent nerve in a hind limb tends to evoke simultaneously two antagonistic reflex effects one of which usually dominates and masks the opposite effect which, though latent, is always present.

3. An attempt is made to analyze these various reflex effects in terms of the nerve impulses of which they are composed, avoiding the postulation of any properties in the central conducting path differing fundamentally from those which have been demonstrated and analyzed in the nerve-muscle preparation.

4. In view of the all-or-none law of nerve conduction the differences in the effects of different afferent stimuli must depend ultimately on differences in the number of afferent fibers stimulated and in the frequency with which each individual fiber is made to respond.

5. Certain facts concerning reflexes require for their explanation the convergence of several afferent conducting paths or their central connections, at a single motor neurone.

6. Proceeding from the basis of these facts an attempt is made to see how far the explanation of reflex inhibition as proposed by Lucas can be applied in the present state of our knowledge to some of the more definitely established facts concerning reflexes. The proposed explanation is based on the analysis by Lucas of so-called Wedensky inhibition. Briefly it is that the impulses follow each other in a conducting path with such frequency that each occurs during the relative refractory period following its predecessor and therefore being subnormal, cannot pass the region of decrement existing at the next synapse without extinction; an internuncial ("pre-motor") neurone being the last link of the chain leading to the motor neurone, has a critical frequency of nerve impulses above which their effect will be inhibitory and below which excitatory.

7. The balancing or algebraic summation of antagonistic central effects described by Sherrington is examined in the light of our present knowledge of the intensity of the afferent stimuli required to produce the effect. This evidence makes it extremely improbable that such balancing can ever be obtained in extensors with inhibitory stimuli strong enough to excite all the fibers in the afferent nerve. This phenomenon, therefore, which has hitherto been regarded as an obstacle to the application of the supposed explanation need not be so considered.

8. The electrical reversal or change from inhibition to excitation on changing the intensity and character of the electrical stimulus applied to an afferent nerve cannot be as simply explained on the basis here considered as appears at first sight. Reflex inhibition can be produced by a single induction shock of moderate intensity, too weak to set up more than a single impulse in each afferent fiber. Therefore if reflex inhibition so produced depends on the frequency of impulses in the pre-motor neurone, this frequency must be set up by convergence of afferent paths or other central connections and not depend directly on the frequency with which the afferent fibers are stimulated.

9. It is suggested that rebound contraction following inhibition may depend on a decrease in the frequency with which impulses converge at the pre-motor neurone when they cease to enter the central mechanism through the afferent fibers. Both rebound and the after-discharge of such reflexes as the crossed extension reflex require an elaboration of central conducting paths great enough to introduce a long delay in the arrival of some impulses at the motor neurones.

10. The conversion of excitation into inhibition by chloroform can be readily explained, as has already been proposed by Lucas, as resulting

from increased decrement at the synapse due to the action of the drug. The observation first described in this paper may be explained as the result of a similar action occurring in some of the individual reflex arcs, together with a change in the frequency of converging impulses such as was invoked to explain rebound.

11. The reciprocal innervation of antagonistic muscles requires appropriate branches so arranged that impulses shall converge at the pre-motor neurones with the proper frequencies to produce excitatory and inhibitory effects.

12. The postural reversal of reflex effect described by Magnus and Sherrington, lends itself well to explanation on the proposed scheme, if we may assume that the proprioceptive impulses on which the effect has been shown to depend are induced by a shortened state of the intramuscular receptor, and arriving at the pre-motor neurone, cause the excitatory frequency of impulses existing therein to be raised above the critical value and become inhibitory.

13. The intrinsic tendency of the spinal centers to exhibit a rhythmic alternation between flexion and extension in absence of afferent stimulation and at a depth of narcosis at which even transection of the spinal cord causes no effect, may be explained by further elaboration of a suggestion by Graham Brown, i.e., that collateral branches are given off whereby impulses may be conducted to the pre-motor neurone of the opposite center and there produce jointly with impulses entering through other paths an inhibitory frequency. The rhythmic alternation would require a still further assumption of a certain measure of synaptic fatigue causing this effect after a brief time to become inoperative and the excitatory frequency therefore to be resumed.

14. The proposed explanations are speculative and more complex than the simple assumption that stimulation of an afferent nerve excites in every instance two antagonistic central effects, but they serve the purpose of demonstrating that so far as we can see at present there are no insuperable obstacles in the way of explaining reflex phenomena on the basis of nerve impulse frequency proposed by Lucas.

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BIBLIOGRAPHY

(1) Sherrington: Integrative action of the nervous system, 1906.
(2) Sherrington and Sowton: Proc. Roy. Soc., 1911, lxxxiv, B, 201.
(3) Sherrington and Sowton: Proc. Roy. Soc., 1911, lxxxiii, B, 435.
(4) Sherrington: Proc. Roy. Soc., 1908, lxxx, B, 53.
(5) Sherrington and Sowton: Unpublished paper on Reflex rebound, 1911.
(6) Forbes: Quart. Journ. Exper. Physiol., 1912, v, 149.
(7) Graham Brown: Proc. Roy. Soc., 1912, lxxxv, B, 278.
(8) Forbes: Proc. Roy. Soc., 1912, lxxxv, B, 289.
(9) Sherrington: Proc. Roy. Soc., 1905, lxxvi, B, 288.
(10) Sherrington and Sowton: Journ. Physiol., 1911, xlii, 383.
(11) Miller and Sherrington: Quart. Journ. Exper. Physiol., 1915, ix, 147.
(12) Martin: Measurement of induction shocks, 1912.
(13) Erlanger and Garrey: This Journal, 1914, xxxv, 403.
(14) Graham Brown: Quart. Journ. Exper. Physiol., 1911, iv, 332.
(15) Graham Brown: Proc. Roy. Soc., 1913, lxxxvi, B, 140.
(16) Graham Brown: Journ. Physiol., 1914, xlviii, 18.
(17) Lucas: The conduction of the nervous impulse, 1917.
(18) Lucas: Proc. Roy. Soc., 1912, lxxxv, 495.
(19) Adrian: Journ. Physiol., 1912, xlv, 389.
(20) Adrian: Journ. Physiol., 1913, xlvi, 384.
(21) Adrian: Journ. Physiol., 1914, xlvii, 460.
(22) Lucas: Journ. Physiol., 1911, xliii, 46.
(23) Forbes and Gregg: This Journal, 1915, xxxix, 172.
(24) Graham Brown: Proc. Roy. Soc., 1913, lxxxvii, B, 132.
(25) Adrian and Lucas: Journ. Physiol., 1912, xliv, 68.
(26) Adrian: Journ. Physiol., 1920, liii, 70.
(27) Lutz: This Journal, 1918, xlv, 507.
(28) Lutz: This Journal, 1918, xlv, 515.
(29) Forbes: This Journal, 1912, xxi, 102.
(30) Adrian: Brain, 1918, xli, 23.
(31) Sherrington: Proc. Roy. Soc., 1908, lxxx, B, 565.
(32) Camis: Journ. Physiol., 1909, xxxix, 228.
(33) Porter: This Journal, xxxi, 1912, 141.
(34) Sherrington: Quart. Journ. Exper. Physiol., 1909, ii, 109.
(35) Forbes and Gregg: This Journal, 1915, xxxvii, 118.
(36) Dreyer and Sherrington: Proc. Roy. Soc., 1918, xc, B, 270.
(37) Owen and Sherrington: Journ. Physiol., 1911, xliii, 232.
(38) Bayliss: Proc. Roy. Soc., 1908, lxxx, B, 339.
(39) Sherrington: Quart. Journ. Exper. Physiol., 1913, vi, 209.
(40) Magnus: Arch. f. d. gesammt. Physiol., 1910, cxxxiv, 545.
(41) Graham Brown: Quart. Journ. Exper. Physiol., 1911, iv, 273.
(42) Sherrington: Proc. Roy. Soc., 1908, lxxx, B, 552.
(43) Lewis and Stöhr: Textbook of histology, 2nd ed., 1913, 159.
(44) Sherrington: Journ. Physiol., 1906, xxxiv, 1.

BLOOD VOLUME STUDIES

V. The Carbon Monoxide Method—Its Accuracy and Limitations

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Many investigators consider the whole subject of blood volume to be in a state of hopeless confusion. Say our critics, how can we think otherwise when one set of observers report, the blood volume of dogs as 10 per cent body weight (dye methods), another as 8 per cent (CO method) and still others as 7 per cent (Welcker method). In this series of papers we submit experimental data which go far to explain these discrepancies and point the way to a blood volume method which is reasonably accurate (5 per cent error). In a word, our experimental data indicate that the blood plasma volume is accurately determined as 5 per cent (5 cc. per 100 gram body weight) by a number of dye methods. Furthermore our data show that the total body hemoglobin is accurately determined as approximately 4 per cent by the CO and Welcker methods. But in our opinion the true *total blood volume* is 5+ 4 = 9 per cent body weight, and not 10 per cent or 8 per cent respectively, calculating the hematocrit as 50 per cent. The *fundamental error, therefore, is the assumption that the ratio of cells to plasma is a constant* in all parts of the circulation. This we believe is contrary to fact. Experimental data and discussion of related facts are submitted below in their proper place (paper VII of this series).

It is not necessary to review the different methods and blood volume values as we can refer to a recent discussion by Salvesen (4). We may note in passing that the dilution blood volume methods of Cohnstein and Zuntz (1) which depend upon injection of isotonic sodium chloride solutions have no further usefulness. The recent experiments of Smith and Mendel (5) demonstrate the rapidity with which isotonic salt solutions leave the blood stream. Much of this solution may disappear

within 5 minutes. Some observations in this laboratory on dogs confirm this work of Smith and Mendel on rabbits.

It has been assumed by some workers that the wide divergence in blood volume values might be explained by differences in technical procedure. We thought it highly desirable that these various methods be used in this laboratory with a uniform technique, using the same healthy animals under controlled conditions. For this reason we have described in much detail the various technical procedures and subjected our methods to a variety of control tests.

The carbon monoxide method is very simple in principle. The CO is inhaled and a sample of blood is withdrawn for analysis to determine the amount of contained CO. This gives the amount of dilution effected by the circulating blood and so the total circulating hemoglobin or red cells. It is assumed that the CO is held only by the hemoglobin of the

TABLE 1

Known amounts of CO mixed with deoxidized blood and quantitatively recovered

EXPERIMENT	CO MIXED WITH BLOOD	READING AFTER PYROGALLIC ACID	READING AFTER COPPER SOLUTION	CO RECOVERED FROM BLOOD
	cc.	*cc.*	*cc.*	*cc.*
I	0.265	0.285	0.02	0.265
II	0.265	0.275	0.02	0.255
III	0.390	0.400	0.02	0.380
IV	0.310	0.330	0.02	0.310
V	0.330	0.345	0.02	0.325
Average.....	0.312			0.307

red blood cells, but there is good reason to believe that the hemoglobin of the red marrow and striated muscles (myohematin) participate in this dilution (see paper VII). The carbon monoxide method was first used by Gréhant and Quinquaud (2), later modified by many others and recently by Van Slyke and Salvesen (4), who report a simple method for the accurate analysis of small amounts of CO in blood. This last method with a few minor modifications has been used in our work.

Method of administering the carbon monoxide. The apparatus used for giving the CO gas corresponds in principle with that arrangement employed by Gréhant and Quinquaud (2), later modified by others, as reviewed in the introduction. The technique in detail is as follows: The gas, made by mixing and gently heating formic and sulphuric acids in tube *A*, figure 1, is first burned at *B* until it gives a bright blue flame. The three-way stopcock of the tube is then turned so that the gas bubbles through the bottles *C*, which contain concentrated NaOH

to remove any sulphur dioxide, carbon dioxide and water present. The carbon monoxide runs into the 100 cc. graduated burette D, previously filled with water and supplied with a leveling bulb, D'. This is connected by one arm of a three-way stopcock with a 50 cc. burette E, also previously filled with water and supplied with a leveling bulb, E'. The gas is run through these burettes and out through the arm of another three-way stopcock to B again, where about 200 cc. are burned, thus thoroughly rinsing out the connections. The two burettes are then filled with gas and all stopcocks closed except the one at A which is turned

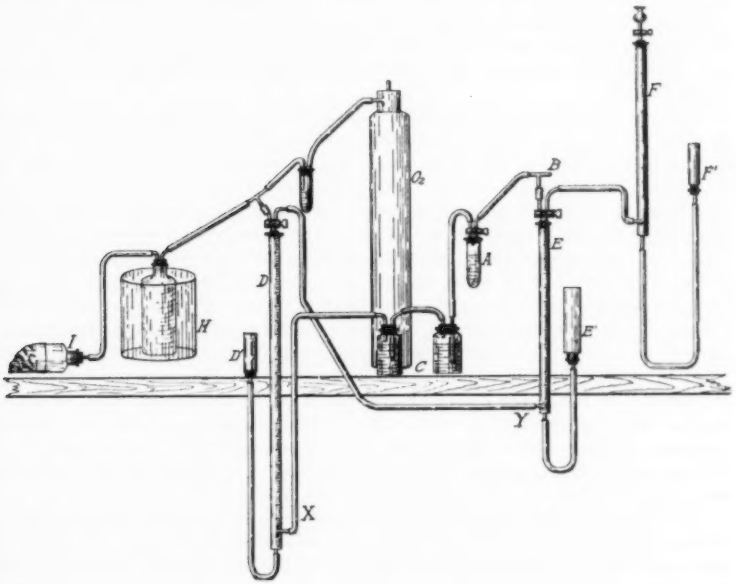

Fig. 1

so as to allow the CO still generating to escape to the outside. The connecting tubes are clamped at X and Y. The gas in E is measured at atmospheric pressure by means of the leveling bulb and 50 cc. are run up into the graduated cylinder F, which contains a solution of copper sulphate in concentrated hydrochloric acid (prepared by Sandmeyer's method). The solution on gentle shaking for a few seconds absorbs all the CO leaving behind the impurities, the volume of which is read directly at atmospheric pressure with the leveling bulb, F'. The gas is then run out of F by raising the leveling bulb and opening the stop-cock. The solution is thus ready for the next analysis.

The respiratory chamber, H, consists of a tank half filled with concentrated NaOH in which stands a bell-jar of about 2000 cc. capacity. The available gas

space is 1000 cc. This is connected with *D* through the other arm of the three-way stopcock and also with the oxygen tank, as shown in figure 1. It is also connected with the nosepiece, *I*. This consists of a glass bottle from which the bottom has been removed, of size varying according to the weight of the dog. A piece of automobile inner tubing of suitable diameter and length is slipped over the bottom of the bottle so as to form a cap for the dog's nose and protect the animal from the glass edge.

To prepare the respiratory chamber the air is sucked up to a mark, leaving about 500 cc. of air in the chamber and the tube to the hood is clamped. Oxygen is then allowed to enter in sufficient quantity to keep a slightly negative pressure within the respiratory chamber. This prevents any outflow of CO during the exhalation. The O_2 makes a smaller respiratory chamber possible and prevents any discomfort to the dog due to deficiency of oxygen. The apparatus is now ready for use.

The dog is tied on its back with the head at the edge of the table. The neck over the jugular vein is shaved and the nose is well greased with crude vaseline, as is also the inside of the nosepiece. This is slipped over the dog's nose to completely cover the mouth and nostrils and held firmly during the inhalation. The tube to the respiratory chamber is immediately unclamped. When all is adjusted, a measured quantity of CO (sufficient to saturate the blood corpuscles one-third if one assume 100 cc. of blood per kilogram body weight, of O_2 capacity 21 cc. per 100 cc. blood) is run in, and the tube rinsed with oxygen. The dog breathes into the respiratory chamber for 6 minutes. During this time as the volume of the gas in the chamber decreases, due to absorption of carbon dioxide, oxygen is supplied from the tank to keep the quantity of gas practically constant.

At the end of 6 minutes the nosepiece is removed. Four minutes later two 5 cc. samples of blood are drawn, by means of a hypodermic syringe, from the jugular vein and placed in tubes with powdered sodium oxalate to prevent clotting. A 10 cc. sample is then taken in a hematocrit tube containing 2 cc. of 1.6 per cent sodium oxalate (isotonic for blood). A sample of air from the respiratory chamber is taken and analyzed as described later.

The purpose of this method is to introduce into the animal's blood stream a known amount of CO. It is therefore essential that there should be no dead space and that the residue of CO left in the respiratory chamber and dog's lungs be accurately determined. The possible dead space is the tube from *D* to *H*. This is eliminated by the inhalations of the dog combined with the flooding of the tube with oxygen. The method employed in analyzing the respiratory chamber air will be discussed shortly. The entire procedure from making the gas to taking the samples is readily carried out and may be completed within 30 minutes.

Method of analyzing the samples of blood. The samples of blood are analyzed in the Van Slyke apparatus for blood gas analysis (6). The method employed is that described by Van Slyke and Salvesen (7). Our technique in detail is as follows: Three cubic centimeters of 0.4 per cent ammonia are introduced into the 50 cc. chamber of a carefully calibrated Van Slyke apparatus. This is followed respectively by 3 drops of caprylic alcohol and 3 drops of a 1 per cent aqueous solution of saponin. Following this 3 cc. more of the ammonia solution are added. The chamber is evacuated and shaken a few times to extract

the air. This is expelled and the process repeated to make certain that all the air is removed and that there is no leak around the stopcocks. On opening the lower stopcock, the solution should rise with a click to the top. Two cubic centimeters of this solution are run up into the cup. A 2 cc. Ostwald pipette, calibrated for complete delivery, is used to introduce the sample of blood into the cup under the ammonia solution. The blood and supernatant liquid are run into the chamber and the cup rinsed with a few drops of ammonia solution. One minute is allowed for laking. One cubic centimeter of a saturated potassium ferricyanide solution is then added, the stopcock sealed with mercury, and the apparatus evacuated until only about 2 cc. of mercury remain in the 50 cc. chamber. The machine is shaken for 15 minutes by a motor which throws the solution gently with a rotary motion against the sides of the chamber, but does not shake so violently as to emulsify the mercury. We found inconsistency in the readings of CO attendant on the variations in the length and severity of shaking, and established this technique after carefully controlled experiments.[1] After taking the apparatus off the shaking machine, the lower stopcock is opened and 1.5 cc. of pyrogallic acid solution (12 grams in 72 cc. of a 33 per cent NaOH solution) are put in the cup and run in a little at a time to absorb the oxygen. When the reading is constant the solution is trapped as completely as possible in the bulb below the lower stopcock, the graduated tube is rinsed clean with water, which also permits the reading of the bottom of the meniscus, previously impossible on account of the turbidity of the solution. That no leak has occurred during the procedure is verified by running in a little of the copper solution. This absorbs all but about 0.025 cc. of the gas. The analysis is duplicated. Previously we have been satisfied to obtain duplicate readings which vary by as much as 0.01 cc. With further experience they now rarely vary more than 0.005.

Discussion of the method of analyzing the blood. The accuracy of this method depends on the technique of the person working with the instrument. To check this we did a set of controls (table 1). About 2 cc. of blood are run into the 50 cc. chamber which is then evacuated and the blood shaken. By this procedure the oxygen in combination with the hemoglobin is removed. This may be run out of the machine at intervals. Within 20 minutes the blood is almost completely deoxidized. The blood is then trapped in the lower bulb and the 50 cc. chamber washed out. The connections are rinsed with CO gas and about 0.2 or 0.3 cc. of CO is introduced and washed in with a few drops of water to empty the bore of the stopcock and give a concave meniscus.

[1] Since sending this paper to press, an article by Theo. K. Kruse in the American Journal of Physiology, Volume lv, number 2, page 289, has come to our notice. In conformity with his results, we find that the long shaking is unnecessary, if, after adding the potassium ferricyanide, and sealing, the solutions are allowed to stand in contact for ten minutes. The apparatus is then evacuated and shaken three minutes longer.

The blood is then released from the lower bulb into the upper chamber and mixed with the gas. With gentle shaking the CO is soon completely absorbed. Six cubic centimeters of the ammonia saponin and caprylic alcohol solution, freed of its gas content in a second Van Slyke instrument, are then poured into the cup and let in to the chamber. The routine analysis is made. As the figures in table 1 indicate, the amount of CO recovered corresponds (within the limit of error of our technique) to that introduced into the machine.

TABLE 2

Blood analyses performed immediately and after 24 hours both with and without oil

EXPERIMENT	SAMPLE DRAWN WITHOUT OIL		SAMPLE DRAWN UNDER OIL	
	At once	After 24 hours	At once	After 24 hours
	cc.	cc.	cc.	cc.
I	0.202	0.200	0.200	0.200
II	0.158	0.160	0.158	0.157
III	0.185	0.180	0.182	0.181
IV	0.238		0.240	
V	0.194	0.190	0.192	
VI	0.180	0.175		
VII	0.190	0.185		
VIII	0.140	0.140		
IX	0.260	0.260		
X	0.123	0.125		

Accuracy in the method of analyzing the blood is, however, useless if gas is lost either in handling the samples of blood or by escape from the blood stream through the lungs or into the tissues. It has been formerly customary to draw the blood under oil and take care that it should not be exposed to the air at any time because of diffusion of the gas. Table 2 shows the result of a series of experiments in which samples drawn under oil are compared with those drawn without oil, both when the samples are analyzed immediately and after an interval of 24 hours. It will be seen that the loss by diffusion from either sample even over a period of 24 hours is not appreciable.

Table 3 shows the result of a series of experiments to determine the interval of time, after removing the nosepiece, which may elapse before the analysis of the blood indicates an appreciable decrease in content of CO. Samples were drawn either while the hood was still on or within 1 or 2 minutes of removing it. Samples were then taken after 4, 7 and 10 minutes. Only the last shows a drop in the reading. We

have, therefore, chosen 4 minutes as a routine length of time to allow complete, even distribution of the gas. Haldane and Smith (3) discuss the possible loss of gas by physical solution in the body fluids. The tension in the blood is so low (0.2 per cent, that supposing all body fluids to be saturated at this same tension the quantity of CO so bound is less than 1 cc.

TABLE 3

Readings of samples of blood drawn at varying intervals after inhalation of CO

DOG NUMBER	WEIGHT	CARBON MONOXIDE LIBERATED BY 2 CC. OF BLOOD			
		1 to 2 minutes	4 minutes	8 to 9 minutes	10 minutes
	kgm.	*cc.*	*cc.*	*cc.*	*cc.*
19–128	27.2	0.200	0.200	0.200	0.190
19–128	27.2	0.175	0.180	0.185	0.175
18–4	18.2	0.160	0.160	0.160	0.155
18–4	18.2	0.194	0.190	0.182	0.178
21–11	29.5	0.195	0.195	0.195	0.195
19–102	18.2	0.205	0.202		0.193
21–6	10.4	0.210	0.210		0.200
Average		0.191	0.191		0.184

Method of calculating the blood volume. To calculate the blood volume, first reduce the volume of gas inhaled to standard conditions by multiplying by the factor $(0.999\text{–}0.0046\ t) \times \frac{\text{barometer}}{760}$ where t is the temperature in degrees centigrade. From this must be subtracted the CO remaining in the respiratory chamber, as determined below.

The reading of the gas obtained from the blood analysis may be converted directly into cubic centimeters of CO per 100 cc. (volume per cent) of blood by using table 1 of Van Slyke's paper on oxygen (8). There is a small amount of nitrogen which is derived from the physically dissolved air in the blood and is read along with the CO after the pyrogallic acid has absorbed the oxygen. So to correct for this 1.2 must be subtracted from the volume per cent derived above as determined by Van Slyke (7). This may either be corrected for as stated, or eliminated as mentioned previously, by absorbing the CO with the copper solution and thus reading directly the true CO volume. Having now the total gas absorbed and the number of cubic centimeters of this recovered in 100 cc. of blood, the former divided by the latter gives the total blood volume. The hematocrit gives the proportion of cells to plasma, while from the weight of the dog the cubic centimeters of blood per kilogram body weight may be calculated. The reading of CO obtained in the Van Slyke apparatus is directly proportional to the cell per cent hematocrit and the blood volume is inversely proportional to the reading. It of course is obvious, therefore, that a hematocrit representative of the entire blood volume is not necessary, but it is necessary that the

sample for the hematocrit and the sample to be analyzed should be taken at the same time so that the per cent of cells in each is the same. If this is the case the true measure of the body hemoglobin will be obtained, though the plasma volume and total volume will be inaccurate according as the hematocrit fails to be representative of the per cent cells in the whole blood.

Analysis of the gas remaining in the respiratory chamber. The principle of the method of analysis employed is to absorb the oxygen from a 100 cc. sample of the gas by means of pyrogallic acid solution. The remaining gas is then mixed with 2 cc. of blood, deoxidized as described before. This oxygen-free blood absorbs the CO which is then determined by the routine analysis of the blood sample. The technic in detail is as follows: A 100 cc. sample of the gas is drawn into a graduated tube of capacity slightly over 105 cc., one end of which is provided with a three-way stopcock, while the other end is connected with a leveling bulb. Mercury is used to adjust the pressure. The tube resembles a urea tube and is graduated only in 5 cc. intervals. Five cubic centimeters of pyrogallic acid are added through one arm of the three-way stopcock and the whole apparatus is shaken at short intervals to facilitate absorption of the oxygen. Meanwhile a little over 2 cc. of blood is deoxidized in the Van Slyke gas apparatus. In about one-half hour the reading in the tube has become constant. The two are connected with rubber tubing and the connections are filled with mercury. As much gas as the analyzer will hold, at atmospheric pressure, is run over and mixed with the blood. Gentle rotating to spread the blood over the walls of the chamber, with remixing of the gas in the tube and Van Slyke apparatus at 3 minute intervals, insures practically complete absorption of the CO within 45 minutes. The blood is then analyzed by the routine technic—the gas-free ammonia solution being introduced as above. An average of those analyses given in table 4, in which the per cent of residual O_2 is approximately 30 per cent, gives, with a calculated total chamber and lung volume of 1500 to 2000 cc. (depending on the size of the dog) approximately 2.00 cc. of unabsorbed CO. The total volume of lungs and respiratory chamber is estimated rather than measured, as by Gréhant and Quinquaud, as the correction would be at most less than 1 cc. of gas, which is a degree of precision the accuracy of the method does not permit.

The limited number of experiments so far performed suggests that the amount of CO remaining in the respiratory chamber is a factor of the per cent of O_2 found in the jar. Thus table 4 shows that the residual CO is doubled or tripled when the per cent of O_2 is between 40 and 50. The amount of O_2 introduced into the respiratory chamber is now regulated so as to constitute approximately 30 per cent of the total volume. Under these conditions the residual CO amounts to about 2 cc., which figure we now use in calculating the volume, as mentioned above.

This method is not the one first employed in the laboratory, nor is it identical to those described in the literature. It has finally been adopted because it is reasonably easy to carry out and may readily be controlled. Two series of controls were run. First, 100 cc. of air

from the room or out of doors were analyzed in the same way. It was found that on an average 0.08 cc. of a gas, which was not absorbed by pyrogallic acid but was absorbed by the copper solution, was obtained. The gas was shown finally to be derived from the pyrogallic acid then in use. Subsequent experiments with other samples of pyrogallic acid gave at most 0.01 cc. of such gas. The second series of controls consisted in measuring in the analyzer a small quantity of CO, 0.2 to 0.3 cc., and

TABLE 4

Analyses of 100 cc. samples of gas from respiratory chamber taken immediately after blood volume determination

EXPERIMENT	DOG NUMBER	O_2 IN RESIDUAL GAS	CO IN 100 CC. GAS	TOTAL RESIDUAL CO
		per cent	*cc.*	*cc.*
I	21–30	20	0.060	1.00
II	21–10	45	0.340	5.61
III	19–102	50	0.317	5.68
IV	21–5	44	0.310	4.65
V	19–128	30	0.110	2.20
VI	18–4	30	0.110	2.03
VII	21-6	30	0.110	1.80

TABLE 5

A known amount of CO mixed with 100 cc. of air. Quantitative recovery of CO by method described

EXPERIMENT	CO MIXED WITH 100 CC. OF AIR	CO RECOVERED IN BLOOD MIXTURE
	cc.	*cc.*
I	0.15	0.140
II	0.21	0.205
III	0.16	0.185
IV	0.31	0.314
Average.....	0.208	0.211

of running this into the tube with 100 cc. of air. This gas was then analyzed by the same procedure as in the case of the respiratory chamber air. The tabulated results in table 5 show that the CO is completely recovered by this procedure.

Hemorrhage control experiments. The following hemorrhage experiments were carried out as a control of the CO blood volume method. If the CO method is accurate it should be possible to determine the hemoglobin volume before and after unit bleedings of known amounts and

show that the measured and calculated hemoglobin values compare with reasonable accuracy. In all these experiments the control determination was made the day previous to the hemorrhage. The second blood volume determination was done immediately following the hemorrhage. The determination of blood volume was made from 4 minutes to 1.5 hours following the completion of the hemorrhage, depending on the condition of the animal after the bleeding. In each instance the blood was drawn by means of a needle in the jugular vein into 1.6 per cent sodium oxalate, measured, and the hematocrit values determined from a 10 cc. sample. Anesthesia was not used in these experiments which cause no discomfort to the dogs except the puncture of the skin by the hypodermic needle.

TABLE 6

Hemorrhage experiment

Dog 19–98. Young, active male. Weight, 55 pounds

DATE 1920	TIME	RED CELLS HEMATOCRIT	BLOOD VOLUME		BLOOD REMOVED			CALCULATED RED CELL VOLUME
			Cells	Plasma	Total	Cells	Plasma	
		per cent	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
February 23	11:30 a.m.	55.9	**974**	768				
February 24	4:30 p.m.	49.4			624	308	316	
February 24	5:30 p.m.	42.2	**678**	928				**666**
February 25	4:30 p.m.	32.3			615	199	416	
February 25	5:15 p.m.	30.8	**535**	1202				**467**
February 26	2:30 p.m.	27.5	**461**	1216				
February 27	1:15 p.m.	25.7	**452**	1307				
February 28	12:00 m.	27.1	**524**	1408				
March 5	2:00 p.m.	34.0	**833**	1616				
March 9	3:30 p.m.	39.0	**856**	1338				

20 cc. whole blood drawn for each determination.

Table 6 presents a typical experiment on a normal dog which was bled large amounts on two successive days. About one-third the hemoglobin volume was removed in each bleeding. The last column gives the expected or calculated hemoglobin or red cell volume. It is noted that the calculated and measured red cell volumes following the first bleeding are practically identical, 666 cc. and 678 cc. respectively. Following the second large hemorrhage, we note the measured cell volume is 60 cc. greater than the calculated cell volume. On the following day the hemoglobin volume has fallen to the expected level and one is inclined to explain the 2nd day's figures as due in part to an error in technique. However, there are physiological variations in these figures

which cannot be discussed at this time but may explain some of these fluctuations. Following the low level of the two days after the hemorrhages, we note a steady gain in hemoglobin volume. It may be noted that the amount of blood removed for each analysis (20 cc.) is allowed for in the bleeding periods and recorded figures. The calculated volume in each instance is obtained by subtracting from the normal cell volume the actual amount of red cells removed during the bleeding and experimental analysis.

TABLE 7

Hemorrhage experiment

Dog 20-37. Young, active, female. Weight, 22 pounds

DATE 1920	TIME	RED CELL HEMATOCRIT	BLOOD VOLUME		BLOOD REMOVED			CALCULATED RED CELL VOLUME
			Cells	Plasma	Total	Cells	Plasma	
		per cent	*cc*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
February 16	3:30 p.m.	50.8	**433**	420				
February 17	3:30 p.m.	50.3			250	126	124	
February 17	5:30 p.m.	39.9	**322**	483				**307**
February 18	4:00 p.m.	30.6	**310**	704				
February 19	4:15 p.m.	29.8	**310**	731				
February 20	2:30 p.m.	30.6	**302**	684				
March 12	7:50 a.m.	55.0	**418**	342				

20 cc. whole blood drawn for each determination.

Table 7 shows a single hemorrhage experiment. The calculated and measured red cell volume correspond very closely, 307 cc. and 322 cc. respectively. Following this bleeding there is not a prompt response nor any appreciable amount of hemoglobin constructed during the 3 days following the large hemorrhage. The daily bleeding of 20 cc. may have disturbed the normal body reaction tending to reproduce hemoglobin and red cells and we must assume that hemoglobin construction was practically stationary. In general we may say that following these large bleedings there was little evidence of very active reconstruction of red cells and hemoglobin during the first period of 2 to 5 days. It is more than probable that the daily bleedings of 20 cc. whole blood plus the CO inhalation actually inhibited the normal reaction which we believe is prompt and productive under normal conditions.

Table 8 shows an experiment very similar to that recorded in table 6. After the first bleeding the calculated and measured red cell volume correspond closely, the measured volume exceeding the calculated by 20 cc. After the second large bleeding we note again the excess of

the measured volume over the calculated hemoglobin volume as 36 cc. This might be explained as a temporary emergency reserve reaction due to mobilization of hemoglobin normally retained in sluggish or isolated sinusoids of the spleen and red marrow. The reaction is similar in all these three experiments. Also there is a fall to the expected value on the days following the hemorrhages. The several days following the two bleedings in the experiment show periodic fluctuations in red cell volume but no significant rise in hemoglobin values. We hope to come back to this point again in subsequent publications.

TABLE 8

Hemorrhage experiment

Dog 19-66. Young, active male. Weight, 29 pounds

DATE 1920	TIME	RED CELL HEMATOCRIT	BLOOD VOLUME		BLOOD REMOVED			CALCULATED RED CELL VOLUME
			Cells	Plasma	Total	Cells	Plasma	
		per cent	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
February 2	4:30 p.m.	59.6	**573**	388				
February 3	3:00 p.m.	58.1			330	192	138	
February 3	3:14 p.m.	56.7	**403**	307				**383**
February 4	4:20 p.m.	35.5			330	117	213	
February 4	5:04 p.m.	34.9	**300**	560				**264**
February 5	3:30 p.m.	25.5	**261**	761				
February 6	3:30 p.m.	26.2	**324**	911				
February 9	12:15 p.m.	30.5	**300**	683				
February 12	3:00 p.m.	32.8	**318**	650				
March 11	2:30 p.m.	52.4	**528**	479				

20 cc. whole blood drawn for each determination.

Table 9 shows an experiment with two large bleedings on consecutive days. This experiment gives the measured values as *lower* than the calculated red cell volumes. We may explain this apparent exception to our other experiments as due to a slight error in the base line or normal hemoglobin values. The difference, however, is not great and the final hemoglobin volume of 179 cc. is very close to the calculated value of 184 cc. This dog, unlike the others, shows a distinct gain in hemoglobin volume in the few days after the hemorrhages.

The experiments in table 10 show how closely the cell volumes agree when the determinations are made on the same animal at different periods. The animals were all young healthy adults. Puppies show certain variations which are being studied at present and will be reported at some future time. In two instances here two determinations

TABLE 9

Hemorrhage experiment

Dog 20–34. Young, active male. Weight, 23 pounds

DATE 1920	TIME	RED CELL HEMATOCRIT	BLOOD VOLUME		BLOOD REMOVED			CALCULATED RED CELL VOLUME
			Cells	Plasma	Total	Cells	Plasma	
		per cent	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
April 13	3:30 p.m.	58.0	**434**	315				
April 14	3:00 p.m.	55.0			260	143	117	
April 14	3:10 p.m.	49.2	**265**	273				**291**
April 15	3:20 p.m.	41.0			260	107	153	
April 15	3:40 p.m.	35.9	**179**	319				**184**
April 16	2:00 p.m.	29.1	**233**	568				
April 20	2:30 p.m.	36.0	**292**	519				

20 cc. whole blood drawn for each determination.

TABLE 10

Blood volume determinations on normal dogs. CO method—red cell volume

DOG NUMBER	DATE	WEIGHT	HEMATOCRIT	RED CELLS	PLASMA	BLOOD PER 100 GRAMS	CELLS PER 100 GRAMS
		kgm.	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*
19–66	December 29, 11:15 a.m.	13.2	63.4	**602**	347	7.21	**4.57**
19–66	December 29, 4:15 p.m.	13.2	60.3	**604**	355	7.28	**4.39**
19–66	February 2	13.2	59.6	**573**	388	7.30	**4.36**
19–137	December 10	12.9	50.6	**540**	528	8.25	**4.17**
19–137	December 11, 3:30 p.m.	12.9	53.4	**563**	491	8.14	**4.37**
19–137	December 11, 4:45 p.m.	12.9	48.9	**567**	593	8.97	**4.39**
19–137	December 16	12.9	50.8	**536**	520	8.16	**4.15**
20–37	February 16	10.0	50.8	**433**	420	8.54	**4.34**
20–37	March 12	10.4	55.0	**418**	342	7.28	**3.68**
21–5	August 6	6.6	46.3	**249**	289	8.16	**4.38**
21–5	August 9	7.0	43.7	**239**	308	7.77	**3.89**
21–5	August 17	7.2	43.3	**244**	320	7.89	**3.94**
21–5	August 18	6.6	43.0	**243**	323	8.60	**4.26**
21–5	September 28	6.9	52.3	**253**	231	6.99	**4.09**
21–5	October 18	7.0	52.4	**253**	230	6.86	**4.02**
21–5	October 21	7.2	50.3	**246**	254	6.84	**3.86**
Average				**454**	389	7.78	**4.20**

were made on the same animal during the same day. In both of these cases there was a slight residue of CO gas remaining in the blood when the second determination was carried out. This was corrected by making a CO analysis with a sample of blood drawn immediately before

determining the second blood volume. The variation in cubic centimeters of blood per 100 grams of animal's body weight is due to the variation in the plasma volume determination. The cell volume varies within experimental limits. In other words, here again is evidence of a reasonably correct cell volume determination by the CO inhalation method as outlined above.

DISCUSSION

We wish to stress the fact that the carbon monoxide method gives a true measure of the pigment substances of the body which hold CO so tenaciously. These pigment substances include the hemoglobin of the circulating blood and probably that in the stagnant sinusoids of the spleen and red marrow. It is very probable that the muscle hemoglobin (myohematin) is also included in these pigment substances which dilute rapidly the inhaled carbon monoxide. We believe then that this CO method gives an accurate measure of this *body hemoglobin* but we fall into decided error when we *calculate* the total blood volume from the red cell hematocrit. The correctness of this calculation depends on the assumption that the blood hematocrit obtained from the jugular vein is representative of all parts of the body circulation, including arterioles and capillaries. We believe these figures obtained by *calculation* are *incorrect for the plasma volume* which is actually much larger than the hematocrit calculation would indicate.

In this paper we use the term *hemoglobin volume* to indicate the pigment substances which are concerned in the dilution of the inhaled carbon monoxide. It is evident that we do not believe that this figure represents only the actively circulating hemoglobin in the red cells but this circulating hemoglobin, of course, makes up the greatest part of the figure (90 to 95 per cent).

The hemorrhage experiments in which unit amounts of blood are removed, the body hemoglobin being measured before and after the single or double bleedings, give strong evidence of the accuracy of the method employed. The calculated and actual measured values correspond very closely. Moreover, repeated determinations on normal dogs under uniform conditions give figures which are quite uniform. All this indicates that the method of estimation is accurate provided we understand the fact that we are measuring only the *body hemoglobin* and *not the plasma volume.*

SUMMARY

The carbon monoxide method gives a reasonably accurate measure of the *body hemoglobin* and *hemoglobin volume*. We use these terms to indicate the total body pigment substances which fix the inhaled CO and bring about its dilution in the circulating blood. It is obvious that the true red cell hemoglobin volume is slightly less than the total hemoglobin volume.

Repeated hemoglobin volume estimations on normal dogs give figures which are reasonably constant, allowing for certain expected physiological variations.

Hemoglobin volume estimations before and after unit hemorrhages give figures for the actual *measured* and indirectly *calculated* amounts which are in close accord

In our opinion this method alone gives no accurate information concerning *plasma volume*.

Various controls of the several steps of this method, as used in our hands, are submitted. These controls establish this method as accurate at least within 5 per cent error. Further refinements and the use of packed red cells, especially in anemia, increase the accuracy of the method.

BIBLIOGRAPHY

(1) Cohnstein and Zuntz: Arch. Gesammt. Physiol., 1888, xlii, 303.
(2) Gréhant and Quinquaud: Journ. de l'Anat. et de Phys., 1882, xviii, 564.
(3) Haldane and Smith: Journ. Physiol., 1899–1900, xxv, 331.
(4) Salvesen: Journ. Biol. Chem., 1919, xl, 109.
(5) Smith and Mendel: This Journal, 1902, liii, 323.
(6) Van Slyke: Journ. Biol. Chem., 1917, xxx, 347.
(7) Van Slyke and Salvesen: Journ. Biol. Chem., 1919, xl, 103.
(8) Van Slyke: Journ. Biol. Chem., 1918, xxxiii, 127.

BLOOD VOLUME STUDIES

VI. Plasma Volume as Determined by Hemoglobin Injection

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The hemoglobin method is exactly like the vital dye method and consists in the introduction intravenously of a known amount of a fresh hemoglobin solution. After a 4 minute period to permit of uniform distribution of the hemoglobin in the circulating blood a sample of blood is drawn into isotonic oxalate. From this plasma sample is determined colorimetrically the degree of dilution of the known hemoglobin introduced and the blood plasma volume is directly measured.

We wish to repeat that this method and many other similar methods give reasonably accurate figures for the blood *plasma volume* but we may not assume that the calculated red cell volume as determined by the hematocrit is also accurate. This point will be elaborated in the following paper.

All our experimental data point to a relatively *constant blood plasma volume* in adult dogs under uniform conditions. Estimated by the hemoglobin or dye methods the normal blood plasma volume of an adult healthy active dog is very close to 4.9 to 5.0 cc. per 100 gram body weight. There are normal variations due to age, physical condition and physiological activities which we hope to review in the near future. At this point we may refer to observations made in this laboratory by means of the dye method (3), (2), (7).

One need not be surprised at certain fluctuations in the plasma volume as determined by the hemoglobin or dye methods. On the other hand there is more reason for surprise at the constant figures obtained under certain conditions. When we contrast circulatory conditions in resting dogs with those of great muscular activity or digestive activity we may well wonder at the mechanism which shuts off one mass of body capillaries as another is opened to great activity. One can im-

agine that the greater part of spleen and red marrow sinusoids may at times be very incompletely washed with circulating plasma or again actively a part of the circulation. There is reason to believe that this constant circulating blood plasma is one factor in itself which is concerned with the partial isolation of one capillary field as another is opened for activity (digestion, for example). The *blood proteins* may be concerned in this adjustment complex and it has been pointed out in publications (4), (5) from this laboratory that the body will not tolerate great reductions in the concentration of the blood plasma proteins; further that the body can form these serum proteins only with difficulty and slowly. If the blood plasma proteins regulate circulating plasma fluctuations we may say that the body tissues which produce or replace the plasma proteins may take part in the regulation of circulating plasma volumes. We hope to supply experimental data which will help to solve these problems.

Some critics say that the dye method may be inaccurate because of the fact that a chemical substance foreign to the body is introduced into the blood stream. The very presence of this dye may modify circulatory conditions although admittedly there is no evidence of any cardio-vascular disturbance. To answer this objection hemoglobin is an ideal substance as it is not foreign to the blood stream and may be given intravenously in considerable amounts to dogs and man without the slightest clinical disturbance. Hemoglobin was first used in this way by Barratt and Yorke (1). The accuracy of the hemoglobin method is largely dependent on our knowledge that no hemolysis of red cells is caused at any step in the procedure. We have controlled this point in our experiments as given below. It is significant that the dye method and the hemoglobin method give identical figures for blood plasma volume.

Method. The general technical procedure in these experiments is identical with that previously described in work on blood plasma volume with vital dyes (3). The hemoglobin solution used in our work is prepared in the following manner: About 12 cc. of blood from the jugular vein of a dog are emptied into each of two or three 15 cc. centrifuge tubes containing about 2 cc. of 1.6 per cent oxalate solution. These tubes are then centrifuged for 30 minutes at 2500 revolutions per minute. After removing the plasma, 6 cc. of the packed red cells are mixed and shaken for 5 minutes with 30 cc. of distilled water. This makes a dilution of 1 cc. of packed cells in 6 cc. of fluid, which is a concentration at which the red cells are completely laked. This solution is clear and contains a negligible amount of stroma which will settle out on centrifugalization or on standing over night. In most of our experiments this hemoglobin solution is not centrifugalized but

allowed to stand over night in the ice chest. Twenty-five cubic centimeters of the hemoglobin solution is the amount found to be satisfactory for determining the plasma volume of an average dog. More or less may be used, depending upon the size of the dog, and the individual taste in color readings.

In testing this method only normal adult dogs were used. A clean hypodermic needle is inserted into the jugular vein, and about 12 cc. of blood drawn in a clean syringe previously rinsed in 0.9 per cent saline. This sample of blood is then emptied carefully without bubble formation to prevent hemolysis, into a 15 cc. graduated hematocrit tube containing 2 cc. of 1.6 per cent sodium oxalate solution. The syringe is then rinsed in 0.9 saline and another sample of blood drawn and emptied into another oxalated hematocrit tube similar to the first. The blood and oxalate are mixed by inverting the tubes twice, which are then stoppered. Twenty-five cubic centimeters of hemoglobin solution, which has previously been measured into a porcelain dish using a calibrated pipette, are drawn up into a large syringe along with 5 to 10 cc. of 0.9 per cent saline used to wash out the porcelain dish. This solution is now injected slowly into the jugular vein and the hemoglobin rinsed out of the large syringe by withdrawing and reinjecting a little blood. The needle which has been in the vein during this process is allowed to remain, while about 10 cc. of saline are slowly injected to prevent clot formation in the needle. Exactly 4 minutes after injection of the hemoglobin solution a sample of blood is drawn in a clean syringe previously rinsed with 0.9 per cent saline, and emptied into a 15 cc. hematocrit tube containing 2 cc. of 1.6 per cent sodium oxalate. Immediately the syringe is rinsed in 0.9 per cent saline and a second sample is collected in a similar manner. Both the tubes are then inverted twice to insure thorough mixing, and stoppered. All four tubes are now centrifugalized at 2500 revolutions a minute for 30 minutes. The total contents and the number of cubic centimeters of cells are then noted. The red cell hematocrit normal values are computed from the control tubes.

The hemoglobin colored plasma is read in a Hellige's colorimeter against a standard prepared as follows:

1. One cubic centimeter of the hemoglobin solution from which 25 cc. were taken above for injection, is pipetted into a clean test tube to which is then added exactly 9 cc. of distilled water, thus making a 10 per cent hemoglobin solution.

2. Five cubic centimeters of the above 10 per cent solution are then diluted with 10 cc. of clear plasma obtained from the first two samples of blood drawn from the dog. Thus 0.5 cc. of the hemoglobin solution is diluted in 15 cc. of the standard.

Against this standard the colored unknown plasma is read and expressed in per cent. In the formula below let this reading be referred to as R. The number of cubic centimeters of hemoglobin solution injected into the dog is referred to as D, and the correction for the dilution by the oxalate present in the tinted plasma as C. Thus C expresses the ratio between the actual concentration of color in the plasma when diluted with 2 cc. of oxalate solution to the value when not so diluted.

The standard for color comparison contains 1 cc. of hemoglobin solution in 30 cc. of fluid.

Therefore D cc. of the hemoglobin solution (amount injected) will impart the same color intensity to 30 D cc. of fluid.

If, however, the color intensity is $\frac{R}{100\ C}$ (i.e., the colorimetric reading in per cent corrected for the dilution of the plasma by the oxalate solution).

Then, the number of cubic centimeters of fluids that have been tinted by D cc. of hemoglobin solution $= \frac{(30\ D)\ (100\ C)}{R}$ or, the *plasma volume* in cubic centimeters $= \frac{3000\ D\ C}{R}$.

Unless otherwise stated all experiments were done upon healthy dogs. A mixed diet of table scraps, including bones, meat, bread, potatoes, rice, etc., was given daily. Water was in the cages at all times.

Method control. If any hemolysis of red cells is produced by any of the steps of this method then the method has little if any value. Dogs' corpuscles are notoriously fragile and all workers in this field should control the method in their hands under local laboratory conditions. For this reason all specimens are drawn in duplicate and all readings of these duplicate specimens must check. Blood should be emptied down the sides of centrifuge tubes as air bubbles often cause slight hemolysis. For the same reason the last 1 to 2 cc. of blood in the syringe which may contain air bubbles should be discarded.

Our solution of hemoglobin is not made isotonic. Does this hypotonic solution by itself cause any hemolysis in the circulation? We controlled this step and others in the method by the injection of distilled water into the vein instead of the hemoglobin solution. In all other respects the blood samples, etc., were handled in the usual fashion described above. Two samples of blood were drawn into isotonic sodium oxalate after a period of 4 minutes following the injection of 25 cc. of distilled water. Six dogs injected in this way with 25 cc. of distilled water showed water-clear blood plasma obtained in the usual way. There was no hemolysis.

Other experiments in which larger amounts of distilled water were injected intravenously show that a very faint trace of hemolysis is observed only when the distilled water injected amounts to approximately 10 per cent of the total plasma volume. It is obvious therefore that we are within safe limits when hypotonic solutions in amounts less than 5 per cent of plasma volume are used for intravenous injection.

Experimental observations. One of the first things to be established for the hemoglobin method, as for the dye methods, is the curve of disappearance of the introduced hemoglobin. It will be noted that the

hemoglobin leaves the circulation less promptly than the vital dyes but unlike the vital dyes is completely removed from the plasma within 18 hours. The body has other methods of dealing with hemoglobin in the circulating plasma as compared with vital dyes which persist in the blood stream for many days, depending in part upon the amount injected (2). For certain purposes this complete removal of the hemoglobin from the blood plasma within 18 hours is of especial importance and makes the hemoglobin method of distinct value for certain experimental work.

TABLE 11

Rate of elimination of hemoglobin solution from circulating blood plasma

DATE 1920	DOG NUMBER	WEIGHT	2 MINUTES	4 MINUTES	10 MINUTES	30 MINUTES	2 HOURS
		kgm.					
February 2	20–80	15.91	100	101	96	92	74
February 17	20–80	18.17	100	99	96	86	75
February 18	20–80	17.95	100	100	94	86	60
February 19	18–4	20.21	100	102	97	96	78
February 20	18–4	20.11	100	100	99	91	
February 25	18–4	20.45	100	98	94	92	78
Average				100	96	91	73

Table 11 gives the figures for the decrease in concentration of the injected hemoglobin in the circulating plasma. In all experiments 25 cc. of the standard hemoglobin solution were injected into the jugular vein. The samples removed at the end of 2 minutes are figured as 100 per cent—all samples in duplicate. It is clear that the hemoglobin remains in the blood stream with a minimal removal during the first 10 minutes. We think it is safe to assume that a 4-minute sample will give values which should be within 2 to 3 per cent of the true dilution if there was absolutely no escape of hemoglobin in the circulation. We consider that this figure is within the accuracy of the colorimetric determination.

After 1 to 2 hours the plasma samples take on a slightly brownish tint which makes accurate color comparisons difficult. This is undoubtedly due to the change of circulating hemoglobin into bile pigment substances (6), which is known to take place rapidly in dogs. This reaction is a recognized body function and makes for a complete and rapid removal of the injected hemoglobin, so that the plasma figures after 18 hours are zero. It is possible by the hemoglobin method to repeat

such blood volume observations at relatively short periods without the troublesome complication of a residual plasma dye.

Table 12 includes many observations on young adult, active, healthy dogs. Several observations on the same animal under different dates are recorded. There are considerable variations in body weight and plasma volume. The dogs were all on a diet of mixed table scraps but no effort was made to insure a constant food intake and body weight in these experiments. The average for this group of dogs is 51.8 cc.

TABLE 12

Blood plasma determination on normal dogs

DATE 1920	DOG NUMBER	WEIGHT	HEMATOCRIT RED CELLS	PLASMA VOLUME	CELL VOLUME	TOTAL BLOOD VOLUME	BLOOD PER KILOGRAM	PLASMA PER KILOGRAM
		kgm.	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
January 22	20–80	17.85	48.6	**921**	871	1792	100.4	**51.6**
January 30	20–80	16.81	47.4	**918**	855	1773	105.5	**54.6**
February 17	20–80	18.17	45.8	**1057**	895	1952	107.4	**58.1**
April 7	20–73	16.36	55.8	**741**	934	1675	102.4	**45.3**
February 9	18–4	19.88	56.7	**928**	1218	2146	107.9	**46.7**
February 19	18–4	20.21	55.4	**993**	1161	2154	106.5	**49.1**
February 20	18–4	20.11	49.1	**1089**	987	2076	103.2	**54.1**
February 25	18–4	20.45	47.0	**1113**	1001	2114	103.3	**54.4**
March 23	18–4	19.08	55.4	**868**	1076	1944	101.9	**45.5**
April 19	18–4	18.40	48.6	**1082**	1020	2102	114.2	**58.8**
August 27	19–102	16.82	50.7	**876**	900	1776	105.6	**52.1**
Average		18.55	50.9	**962**	992	1954	105.3	**51.8**

plasma per kilo body weight. Calculated blood volume on the basis of a 50 per cent hematocrit introduces the error already referred to. These figures correspond to those recorded in this laboratory with the brilliant vital red method.

Table 13 is very much like the preceding table but these dogs are all much heavier. It is a constant observation in this laboratory that these large, heavy, quiet dogs give lower plasma volumes than do the active, muscular dogs of smaller size. A part of this plasma volume decrease undoubtedly is due to the increase in fat which is less rich in capillaries than other body tissues like muscle. The plasma volume, therefore, may be expected to decrease per kilo as a dog puts on fat and becomes less active. Our highest blood volume figures per kilo are recorded in lean, very active, young adult dogs. The blood plasma figures are apt to be more constant in the larger dogs—see dog 19-128, table

13. Compare the average plasma volume per kilo = 51.8 cc. for the active dogs, and the plasma volume per kilo = 41.0 cc. for the large, fat dogs (table 13).

TABLE 13

Blood plasma determination on normal large dogs

DATE 1920	DOG NUMBER	WEIGHT	HEMATOCRIT RED CELLS	PLASMA VOLUME	CELL VOLUME	TOTAL BLOOD VOLUME	BLOOD PER KILOGRAM	PLASMA PER KILOGRAM
		kgm.	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
March 17	19–128	28.17	55.5	**1064**	1339	2403	85.4	**37.7**
March 25	19–128	27.85	56.3	**1099**	1416	2515	90.3	**39.4**
April 7	19–128	28.52	55.5	**1080**	1349	2429	85.1	**37.8**
April 29	19–128	27.40	57.2	**1136**	1518	2654	96.8	**41.4**
June 7	19–128	26.70	60.5	**1143**	1749	2892	108.3	**42.8**
July 13	19–128	27.70	62.5	**1122**	1872	2994	108.1	**40.5**
July 20	19–128	27.50	61.6	**1137**	1824	2961	107.7	**41.3**
April 14	17–160	26.92	60.5	**1143**	1752	2895	107.4	**42.4**
June 24	17–160	24.18	50.4	**1159**	1179	2338	96.7	**47.8**
June 21	18–60	24.10	52.9	**976**	1095	2071	86.0	**40.5**
August 20	17–154	23.20	57.8	**995**	1345	2340	100.8	**42.8**
August 27	21–11	29.21	59.1	**1149**	1661	2810	96.2	**39.3**
Average		26.78	57.4	**1099**	1509	2608	97.4	**41.0**

TABLE 14

Daily blood plasma determination

DATE 1920	WEIGHT	HEMATOCRIT RED CELLS	PLASMA VOLUME	CELL VOLUME	TOTAL BLOOD VOLUME	BLOOD PER KILOGRAM	PLASMA PER KILOGRAM
	kgm.	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
Experiment A. Dog 18–4. Wire-haired mongrel. Lean and active female							
June 14	19.6	50.2	**1057**	1068	2125	108.7	**53.9**
June 15	19.6	48.0	**1100**	1053	2153	110.1	**56.1**
June 16	19.6	48.0	**1084**	1003	2087	106.8	**55.3**
June 17	19.6	45.8	**1220**	1032	2252	115.1	**62.2**
Experiment B. Dog 17–160. Large mongrel bull. Fat and inactive male							
June 14	25.3	53.6	**1120**	1294	2414	95.9	**44.2**
June 15	25.2	48.9	**1138**	1100	2238	88.8	**45.2**
June 16	25.1	48.5	**1203**	1134	2337	93.2	**47.9**
June 17	24.6	52.1	**1163**	1265	2428	98.9	**47.2**

Table 14 gives observations on two normal dogs with daily blood plasma determinations for 4 days. The dogs were fed the usual mixed diet in the afternoon after the blood volume determinations had been

completed. They had access to water at all times. The lighter an more active dog has a higher blood plasma volume per kilo and shows the greater daily fluctuations. These daily bleedings of 20 to 30 cc. cause a slight upset in the red blood cell hematocrit values and may be in part responsible for the variations in plasma volume figures.

SUMMARY

The *hemoglobin method* gives a reasonably accurate measure of the *circulating blood plasma*. Fresh standard hemoglobin solutions can be prepared easily and when used with sufficient care will give uniform figures which are identical with those obtained by the common vital dyes used in blood volume work. The hemoglobin is completely removed from the blood within 18 hours, which may be of advantage in certain experiments.

Observations here recorded show that large, fat dogs present a plasma volume per kilo which is uniformly lower than the figures for lean, active, adult dogs.

In our opinion this method gives no direct information concerning red cell or hemoglobin volume.

BIBLIOGRAPHY

(1) Barratt and Yorke: Proc. Roy. Soc. London, 1909, lxxxi, 381.
(2) Dawson, Evans and Whipple: This Journal, 1920, li, 232.
(3) Hooper, Smith, Belt and Whipple: This Journal, 1920, li, 205.
(4) Kerr, Hurwitz and Whipple: This Journal, 1918, xlvii, 370.
(5) Smith, Belt and Whipple: This Journal, 1920, lii, 54.
(6) Whipple and Hooper: Journ. Exper. Med., 1913, xvii, 612.
(7) Whipple, Hooper and Robscheit: This Journal, 1920, liii, 151.

BLOOD VOLUME STUDIES

VII. Comparative Values of Welcker, Carbon Monoxide and Dye Methods for Blood Volume Determinations. Accurate Estimation of Absolute Blood Volume

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A review of the many blood volume methods and a consideration of the many apparent contradictions in these blood volume figures made it clear to us that a detailed study of these various methods was desirable. We hope to throw some light on many of these vexed questions so that finally we may come to understand the factors which regulate the volume of the circulating blood and recognize the limitations of the various methods. We submit the data of these experiments with the conviction that these figures obtained under uniform conditions, using the same animals and a controlled technic, are of greater value than the data to be obtained in the literature. The methods of different investigators are often incompletely described and may present gross errors—for example, the use of hypotonic oxalate solutions or dry powdered oxalate to prevent blood coagulation may introduce errors into the plasma volume figures which may exceed 10 per cent (1).

In brief, it is our conviction that the *dye or hemoglobin methods give the true plasma volume* (4.8 cc. per 100 grams of body weight), that the *carbon monoxide method gives the true body hemoglobin volume* (4.2 cc. R. B. C. per 100 grams body weight), and finally that the true blood volume is the sum of these two figures (9 cc. per 100 grams of body weight). Hematocrit figures introduce errors when used to calculate the plasma or red cell volume because of the assumption that the ratio of cells and plasma is a constant for all parts of the circulation. This error may become still larger in abnormal conditions where the ratio of cells to plasma may fluctuate more than normal in different areas of the circulation.

For several years these blood volume problems have been of interest to a considerable number of workers in this laboratory. These studies developed as a part of an investigation into the problem of hemoglobin regeneration after simple anemia. Some of these experiments have been published (2). In the course of this work certain defects in the blood volume method of Keith, Rowntree and Geraghty (3) were discovered and modifications were proposed by Hooper, Smith, Belt and Whipple (1). A variety of dye substances was tested by Dawson, Evans and Whipple (4). An acacia blood plasma volume method was described by McQuarrie and Davis (5) and certain advantages of this method pointed out. This critical study by many different workers of these various methods, their limitations and advantages, has been of great value in the development of an accurate and controlled technic.

In all of these methods the same principle is employed,viz., a known amount of the solution (dye, hemoglobin or acacia) is injected intravenously and adequate time is allowed for the complete admixture of this substance with the blood. At the end of this period a sample of blood is withdrawn into an isotonic oxalate solution and centrifugalized to remove the blood cells. The concentration of the injected substance in the supernatant plasma is then determined. It is now easy to compute the volume of plasma required to dilute to this degree the total mass of the substance injected. The original articles should be consulted for details of these methods. We wish to emphasize the fact that in theory all of these methods rest upon the same basis, viz., dilution of the injected substance by the blood *plasma*. For this reason these methods should be termed *plasma volume methods* rather than *blood volume methods*. A fact of equal importance is that these methods agree in showing that in normal adult dogs there are about 4.8 cc. of plasma per 100 grams of body weight.

The carbon monoxide method for the determination of blood volume has been the subject of study by Arnold, Carrier, Smith and Whipple (6). In its essential features their method is similar to that of Salvesen (7). They have carried out many controls on the method and have suggested some alterations in the method of Salvesen. The details of the method need not be repeated. In brief, the animal is forced to breathe into an air-tight but elastic chamber containing oxygen together with a measured amount of carbon monoxide. The carbon monoxide is drawn into the lungs along with the oxygen where it is absorbed almost quantitatively by the hemoglobin of the blood which passes continuously through the vessels of the lungs. The measured

amount of carbon monoxide is thus transferred from the respiratory chamber to the hemoglobin of the circulating blood. A sample of blood is now drawn and its carbon monoxide content determined. The carbon monoxide concentration in this sample furnishes an index of the total quantity of hemoglobin in the body. It will be noted that whereas the other methods mentioned above indicate the *volume of plasma*, the carbon monoxide is taken up into the hemoglobin and thereby furnishes us with an index of the *total body hemoglobin*.

For purposes of further comparison of methods we have used a modification of the direct blood volume method first employed in 1854 by Welcker (8). The method consists essentially in determining the amount of hemoglobin which can be washed out of the animal body. Various modifications and improvements of Welcker's method have been made in the last 50 years. Some of these changes have made possible the more complete removal of the hemoglobin from the body. Others involve the use of the more recently devised methods of estimating the amount of hemoglobin once it is washed out of the body. The method as used by us is described in detail below By means of this method, as in the carbon monoxide method, the blood constituent primarily measured is the hemoglobin. With certain qualifications to be mentioned later, either of these two methods enables one to determine fairly accurately the *total red blood cell volume*, for the hemoglobin of the circulating blood is normally confined entirely to the red blood cells and for each animal there is a definite ratio between the hemoglobin and the red blood cells. These two methods should be termed *body hemoglobin* or *red blood cell volume methods*.

In the experiments to be presented below, blood volume determinations were carried out on each of a series of normal dogs, first by the dye method, shortly thereafter by the carbon monoxide method, which was in turn followed by a determination by a modification of the Welcker method. In this way an excellent opportunity is afforded to study and compare these three methods under practically identical conditions of experimentation.

Method. Adult dogs maintained on a mixed diet were used in all experiments. All were sound, healthy animals unless otherwise noted. Free access to water obtained at all times. Food was withheld for a period of about 12 hours prior to the carrying out of the three blood volume determinations. This was done in order that the plasma might be clear and free from fat.

In carrying out the dye method the procedure previously outlined by Hooper, Smith, Belt and Whipple (1) was followed in all respects. Following this, usu-

ally within a few minutes, a determination by the carbon monoxide method as outlined by Arnold, Carrier, Smith and Whipple (6) was carried out. For each of these determinations approximately 20 cc. of blood were drawn. Preparations were now made for the determination by the third method, i.e., the modified Welcker method. The animal was placed under complete ether anesthesia. A cannula was inserted into one of the larger arteries (carotid or femoral) in such a manner that blood could be withdrawn at will. The animal was bled at a moderately rapid rate from this artery. In order to prolong life and permit the heart to pump out a major portion of the circulating blood cells, warm saline was injected into the vascular system through a cannula inserted into one of the larger veins. In all cases the saline was thus infused into either the femoral or external jugular vein. The fluid was injected at a rate approximately equal to that at which the blood was being withdrawn from the artery. In most cases the heart's action ceased after this exchange of saline for blood had been continued for about 20 minutes.

In order to wash from the vascular system as completely as possible the remaining traces of blood, the inflow cannula was changed and the saline was forced under pressure into the arterial system. The fluid thus artificially forced to circulate was in great part recovered from the right side of the heart by means of a cannula inserted and tied into the right auricular appendage in such a way as to drain the fluid collecting in the right auricle. In several of the earlier experiments a part of the saline forced into the arterial system found its way back past the aortic valves into the pulmonary circuit, and forcing its way into the bronchi escaped through the nose. In subsequent experiments this was prevented by clamping a heavy hemostat about the aorta just distal to the aortic cusps during the period of artificial perfusion. The perfusate was thus prevented from backing up into the pulmonary circulation.

During the period of perfusion the inflow of saline was occasionally interrupted for a number of seconds, for the work of Larson and Bell (9) indicates that intermittent perfusion is more efficacious in washing blood cells out of the vascular system than is a steady continuous flow. To further aid in adequately washing out of the smaller vessels general massage of the animal body was practiced. In most cases the artificial perfusion was continued for about 1½ hour. The fluid recovered during the last part of this period appeared almost as clear as water and on centrifugalization such samples were found to contain very few blood cells indeed. At autopsy only scattered traces of macroscopic blood could be made out. These findings are described more in detail in individual experiments. The relative freedom of the vessels from blood as shown by microscopic study of stained sections will also be shown later.

All samples of blood recovered from the animal were received into oxalate to prevent clotting. The blood withdrawn before the death of the animal was collected separately in one container. The blood collected by artificial perfusion was also collected separately, usually in two portions, the last portion being of relatively large volume (5 to 15 liters) but containing as a rule less than 10 per cent of the total hemoglobin recovered from the animals. Each sample thus collected was measured and its hemoglobin content determined by the method of Palmer (10). The total number of grams of hemoglobin removed was thus ascertained.

In spite of the prolonged artificial perfusion a certain small part of the total hemoglobin remains behind in the body. In order to measure the total amount of the pigment which perfusion failed to remove we have followed the practice of grinding up and extracting from the various organs and tissues their content of pigment. The skin in all cases was stripped off and being almost free of pigment was discarded. The skeletal muscles together with the fascia were dissected free from the skeletal system, ground up finely in a meat grinder, mixed with water and stirred frequently for a period of about 6 hours. The fluid and particles of muscle were separated in a meat press. A sample of the fluid obtained was further freed of debris by centrifugalization, after which its hemoglobin content was estimated. The parenchymatous organs were separately and individually treated in a similar manner. The gall-bladder with its contents was previously dissected free and discarded. The bile and fecal matter were carefully washed out of the gastro-intestinal tract before this tissue was ground up for extraction. The bones were broken up finely with a hatchet before their extraction was attempted. It is recognized that only a part of the total pigment thus extracted is true red blood cell pigment. Another portion is extravascular pigment, particularly muscle hemoglobin. For this reason the organ extracts are charted separately in all tables. The question as to how much of this pigment can properly be considered to be blood pigment will be discussed later.

The quantity of *blood hemoglobin* recovered by bleeding and perfusion is contained wholly within the red blood cells washed out. Knowing the total number of grams of hemoglobin, the total number of cubic centimeters of red blood cells can be learned. To do this the hemoglobin content of a given amount of red blood cells is determined by reading the hemoglobin content of an initial sample of blood from the same animal and taking into account the ratio of red blood cells to plasma (hematocrit). These figures give us a factor by which the total hemoglobin washed out may be multipled in order to show the total volume of red blood cells recovered. This same factor may be used to convert the hemoglobin figure into red blood cell volume in the case of the carbon monoxide method.

The hemoglobin and red blood cell volume figures are thus given in a very direct and relatively trustworthy manner by the carbon monoxide method or Welcker method. In like manner the dye method being essentially a plasma volume method indicates with fair accuracy the total volume of plasma. In tabulating our results we have placed in bold type those values for whose determination each method is especially adapted. This for the dye method includes plasma volume; for the carbon monoxide or Welcker methods, the total hemoglobin and red blood cell volume. All other figures tabulated are derived indirectly from these on the basis of the hematocrit. As will be explained later,

we have no assurance that the sample of blood centrifugalized (hematocrit) is representative of the entire blood. The figures based on the hematocrit are therefore open to criticism. Our method of tabulation enables one to distinguish these figures from those which are not open to this criticism and are placed in bold type.

Experimental observations. The general plan of experimentation has already been outlined. The results of 14 experiments are presented below. Several others which were incomplete in some important respect are omitted. Experiment 339 may be regarded as a typical experiment. The procedure followed is presented in detail as illustrative of the method used in all other experiments. In this experiment as in most of the others, glucose was added to the saline infused. Our experience indicates that if this be done the heart continues to beat for a longer time and a larger proportion of the blood is expelled before death occurs. This is to be desired because under conditions of artificial perfusion of dying tissues the vascular conditions are altered and circulation in the more remote parts of the vascular tree is sluggish. In addition the phenomenon of intravascular clotting following death may in some cases prove to be a disturbing factor. However, if artificial perfusion is relied upon to remove only the last traces of blood, such disturbing factors are of relatively slight importance. In this experiment the perfusion was continued for nearly 2 hours. During the greater part of this time the perfusate recovered was but slightly blood-tinged. During the last 30 minutes it was almost as clear and colorless as water. If 15 cc. of such a sample be centrifugalized only a very small layer of blood cells will be found in the bottom of the tube. In all 8 grams of hemoglobin were recovered through artificial perfusion, whereas 184 grams in all were recovered through bleeding and perfusion combined. Thus only 4.3 per cent of the total was recovered by perfusion.

Experiment 339. Dog 20-92. Adult male mongrel terrier, in good condition. Weight, 15.45 kgm. Blood volume determination by dye method at 7:37 a.m.: by the carbon monoxide method at 7:41 a.m. Ether anesthesia at 9:40 a.m. in preparation for carrying out the modified Welcker determination. A cannula was tied into the carotid artery. Bleeding from this cannula was commenced at 9:45 a.m. Simultaneously and at approximately the same rate Locke's solution containing 1 per cent glucose and warmed to body temperature was injected through a cannula into the external jugular vein. About 3500 cc. of this fluid were injected within a space of about 20 minutes. In the same period 3430 cc. of blood were withdrawn from the carotid artery. The heart continued to beat until nearly the end of this period at which time the blood flowing out was almost colorless. In order to wash from the vascular system as completely as possible

the remaining traces of blood, saline was forced under pressure into the arterial system by way of the carotid. The fluid thus artificially forced to circulate was recovered from the right side of the heart by means of a cannula inserted through the right auricular appendage into the right auricle of the heart. During the period of artificial perfusion the inflow of saline was occasionally interrupted for a period of a minute or so. To further aid the perfusate in reaching and adequately washing out all of the smaller vessels, general massage of the animal body was practiced. The perfusion was discontinued at 11:10 a.m. after having been in effect nearly 2 hours.

At autopsy the peritoneal and thoracic cavities contain a small amount of clear blood-tinged transudate. No blood is found in the cavities of the heart.

TABLE 15

Experiment 339. Dog. 15.45 kgm. Hematocrit 50.1 per cent red blood cells

	TOTAL HEMOGLOBIN	TOTAL R. B. C.	BLOOD	PLASMA	PER 100 GRAMS BODY WEIGHT			
					Hemoglobin	R. B. C.	Blood	Plasma
	grams	*cc.*	*cc.*	*cc.*	*grams*	*cc.*	*cc.*	*cc.*
Dye method	277.0	857.0	1710.0	**838.0**	1.79	5.55	11.07	**5.42**
Carbon monoxide method	**223.0**	**690.0**	1378.0	675.0	**1.44**	**4.47**	8.92	4.37
Welcker method								
Total bleedings	**184.0**	**568.0**	1133.0	555.0	**1.19**	**3.68**	7.33	3.59
Extracts								
Heart	**0.23**	**0.70**	1.40	0.69	**0.0015**	**0.0045**	0.0091	0.0045
Spleen	**0.26**	**0.80**	1.60	0.78	**0.0017**	**0.0052**	0.0104	0.0054
Skeletal muscles	**22.8**	**71.0**	141.0	69.0	**0.147**	**0.459**	0.913	0.447
Bones	**3.32**	**10.3**	21.0	10.0	**0.021**	**0.067**	0.133	0.065
Total extracts	**26.6**	**83.0**	165.0	80.0	**0.171**	**0.536**	1.066	0.522
Total (including extracts)	**211.0**	**651.0**	1298.0	635.0	**1.36**	**4.22**	8.40	4.11

Lungs, liver, kidneys, gastro-intestinal tissues contain only trace of hemoglobin.

The myocardium is pale brown. The lungs are crepitant throughout, are pale grey and quite bloodless. The spleen appears quite normal. The liver is uniformly brown. The lobulation is very indistinct. No blood visible anywhere. The mucosa of the esophagus is pink. The entire gastro-intestinal tract appears quite bloodless. The pancreas, kidneys and pelvic organs contain no macroscopic blood whatsoever. The bone marrow (femur, ribs) is of a moderate bright red color.

In microscopic sections no red blood cells are seen in the heart, lungs or liver, and only an occasional one can be found in the pancreas. The spleen is quite rich in red blood cells; none whatsoever are found in the kidneys or gastro-intestinal tract. No red blood cells could be found in the skeletal muscle even after prolonged search.

The hemoglobin content of the samples recovered was now determined. From the carotid artery 3430 cc. of blood were recovered before the death of the animal. This was found by the method of Palmer to contain 167 grams of hemoglobin. To this must be added 9 grams of hemoglobin which were contained in the 55.6 cc. of blood drawn as samples for the dye and carbon monoxide method. A total of 11,400 cc. was recovered during the period of artificial perfusion. In this there were 8 grams of hemoglobin. The sum of these quantities (184 grams) is entered in the table under the heading of "total bleedings." (See table 15.)

The skeletal muscles were stripped of and ground up finely in a meat grinder. Total weight, 6920 grams. This mass of ground muscle was thoroughly mixed up and to 500 grams 1500 cc. of distilled water were added. This was frequently and vigorously stirred for a period of several hours. By use of the meat press 1350 cc. of hemoglobin-tinged liquid were recovered. It is certain that there was more fluid which could not be forced out with the press. By a more powerful press we could undoubtedly have removed at least 1500 cc. (i.e., an amount corresponding to the quantity of water added for extraction). A sample of this fluid was further clarified by centrifugalization (30 minutes at 3000 revolutions a minute). To 100 cc. of this fluid 0.4 cc. of concentrated ammonia solution was added. Carbon monoxide was bubbled through and the solution was compared in a Duboscq colorimeter with a standard prepared as in the method of Palmer. This extract fluid was found to contain 0.11 gram of hemoglobin per 100 cc. At this rate 1500 cc. would contain 1.65 grams of hemoglobin. Had the entire 6920 grams of skeletal muscle been extracted instead of only 500 grams we should have recovered 22.8 grams of hemoglobin. The other organs and tissues were treated in the same manner. The bones were broken up finely with a hatchet before being extracted. In all cases the macerated tissues remaining behind in the meat press were quite pale, thus indicating that the extraction of hemoglobin had been quite complete.

A sample of blood drawn from the dog at 7:32 a.m. contained 50.1 per cent red blood cells as was shown by a thorough centrifugalization of a 10 cc. sample of blood drawn into a 15 cc. hematocrit tube containing 2 cc. of isotonic sodium oxalate (1.6 per cent (1)). Another sample of whole blood drawn at the same time was found to contain 16.20 grams of hemoglobin per 100 cc. Since the red blood cells constitute but 50.1 per cent of the volume of this blood it is easily seen that 100 cc. of packed cells would contain nearly twice this amount—32.3 grams per 100 cc., to be exact. This is equivalennt to saying that 1 gram of hemoglobin is contained in 3.09 cc. of red blood cells. At this rate 184 grams of hemoglobin (the amount in the "total bleedings") represent 568 cc. of red blood cells. Since there are 100 parts of blood for every 50.1 parts of red blood cells (as shown by the hematocrit) it follows that 568 cc. of red blood cells represent the quantity of red blood cells contained in 1133 cc. of blood. The difference between 1133 and 586 represents the volume of plasma plus that of the white blood cells. In this experiment the white blood cells comprised about 0.9 per cent of the total blood, as was shown by the hematocrit. The red blood cells plus the white blood cells, then, comprise 51.0 per cent of the blood. The other 49 per cent is plasma. Forty-nine per cent of 1133 is 555. This represents the total plasma in the "total bleedings." The organ extracts may be figured in the same manner with the results shown in table 15.

In the total extracts 26.6 grams more of hemoglobin were recovered. We are convinced that for practical purposes the pigment is completely extracted by our technic. A second extraction of the macerated bones was attempted in experiment 339, but as is noted in the protocol very little additional hemoglobin was dissolved out. Ordinarily a single extraction of finely macerated skeletal muscle leaves the muscle debris quite pale. Apparently by far the greater part of the pigment is removed. A second extraction of much muscle debris was made in a number of cases. A typical result is shown in experiment 349, table 16. In this case 15.9 grams were removed by the first extraction and but 2.0 grams by the second extraction. After the second extraction the muscle tissue was almost perfectly white. Similar extractions made in other organs and tissues show essentially identical results. Repeated extraction of such organs and tissues show that by far the greater portion of the pigment is removed by the first extraction, provided that to the macerated tissue at least an equal part of water be added for purposes of extraction.

Experiment 349. Dog 19–71. Adult male mongrel shepherd. For several weeks has been somewhat less active than normal and is somewhat undernourished but on the whole is in fairly good condition. Weight, 16.36 kgm. Blood volume determination by dye method at 8:40 a.m.; by the carbon monoxide method at 9:01 a.m. Bleeding and perfusion under ether anesthesia in the usual manner begun at 9:20 a.m. Warm 0.9 per cent saline to which was added 1 per cent of glucose was used for infusion. Following death of the animal the tissues were perfused with plain 0.9 per cent saline warmed to body temperature. A total of 24,580 cc. of blood-containing perfusion fluid was recovered.

At autopsy the thoracic organs appear to be almost perfectly free of blood. The spleen is normal in size and appearance. The liver is dark brown, the lobulation is distinct and no blood can be seen even at the centers of the lobules. The stomach appears to be bloodless. The mucosa of the upper part of the small intestine is faintly pink. The tips of some of the rugae of the mucosa of the large intestine are reddened. The pancreas, kidneys and pelvic organs are free of macroscopic blood. The bone marrow is bright red.

Microscopically but few red blood cells are seen in sections of myocardium. The lung contains a moderate number. As usual the spleen is quite rich in blood cells. A few are seen in the vessels in the hepatic spaces. Sections of pancreas are free of blood. A moderate number of red blood cells is found in the mucosa of the stomach and many in the mucosa of the small intestine. On the other hand sections of the large intestine and kidney are free of red blood cells. An occasional red blood cell is to be found in sections of skeletal muscle.

From the carotid artery 3580 cc. of blood were recovered before the death of the animal. This was found to conatin 158 grams of hemoglobin. In addition to this 11 grams of hemoglobin were contained in the 83 cc. of blood drawn as samples. A total of 21,000 cc. of perfusate recovered during the period of arti-

ficial perfusion contained 22 grams of hemoglobin. The skeletal muscles (7136 grams) were ground up, mixed thoroughly and a 200-gram sample extracted in the usual manner with 400 cc. of water. After pressing out the fluid the muscle debris was quite pale. To this debris 200 cc. of water were added and the mixture frequently and thoroughly mixed for several hours. Had the entire mass of skeletal muscle been extracted instead of only 200 grams we should have obtained by the first extraction 15.9 grams of hemoglobin and only 2.0 grams by the second extraction.

The other organs were extracted in the usual manner. By the first extraction the bones yielded 4.8 grams of hemoglobin; by the second extraction, 0.8 gram.

TABLE 16

Experiment 349. Dog; 16.36 kgm. Hematocrit 46.4 per cent red blood cells

	TOTAL HEMOGLOBIN	TOTAL R. B. C.	BLOOD	PLASMA	PER 100 GRAMS BODY WEIGHT			
					Hemoglobin	R. B. C.	Blood	Plasma
	grams	*cc.*	*cc.*	*cc.*	*grams*	*cc.*	*cc.*	*cc.*
Dye method	218.0	822.0	1772.0	**932.0**	1.33	5.02	10.83	**5.69**
Carbon monoxide method	**209.0**	**729.0**	1571.0	826.0	**1.28**	**4.46**	9.60	5 05
Welcker method								
Total bleedings	**191.0**	**664.0**	1430.0	752.0	**1.17**	**4.06**	8.74	4.60
Extracts								
Heart	**0.24**	**0.84**	1.81	0.95	**0.0015**	**0.0051**	0.011	0.0058
Spleen	**0.13**	**0.46**	1.00	0.53	**0.0008**	**0 0028**	0.0061	0.0032
Skeletal muscles	**15.9**	**55.0**	119.0	63.0	**0.097**	**0.34**	0.73	0.39
Bones	**4.8**	**17.0**	36.0	19.0	**0.029**	**0.10**	0.22	0.12
Total extracts	**21.1**	**73.0**	158.0	83.0	**0.128**	**0.45**	0.97	0.52
Total (including extracts)	**212.0**	**737.0**	1588.0	835.0	**1.30**	**4.51**	9.71	5.12

Lungs, liver, kidneys and gastro-intestinal tissue contain only traces of hemoglobin.

Additional evidence of the readiness with which hemoglobin can be separated from macerated tissues is furnished by an experiment in which we added a known amount of hemoglobin (in the form of blood) to a weighed sample of macerated skeletal muscle. The mixture was thoroughly stirred after which its hemoglobin was extracted in the usual manner. Analysis showed that we recovered by a single extraction practically all of the added hemoglobin as well as the quantity of pigment which the muscle was known to contain before the artificial addition of hemoglobin.

The freedom of the perfused tissues from red blood cells as shown by microscopic study is well illustrated in experiment 339. Almost complete absence of red blood cells in all sections was noted. In sections from normal unperfused dogs many red blood cells are to be seen. Our experience has been that as a rule macroscopic appearance of the tissues as regards its blood content is well borne out by the microscopic findings. Experiment 351 also serves to illustrate this point. This experiment represents one in which we were particularly *unsuccessful* in perfusing the organs free of blood. The mucosa of the small intestine was brick-red in color and the abundance of red blood cells was quite strikingly shown in microscopic sections of this tissue. The large intestines were free of macroscopic blood and in confirmation no red blood cells whatever could be found microscopically. In this connection it will be recalled that in experiment 339 the absence of macroscopic blood in the entire gastro-intestinal tract was confirmed by microscopic study. The microscope furnishes, we believe, a rather delicate control on the completeness of the removal from the tissues of red blood cells. This is particularly fortunate since in certain tissues, such as skeletal muscle or myocardium, it is impossible to determine in gross just how much of the tissue color is due to residual blood and how much is due to pigments peculiar to the tissues themselves. It is important not to confuse the pigment normally contained within the muscle cells (muscle hemoglobin) with blood pigment. In none of our series was it possible to demonstrate more than a very occasional red blood cell in sections of skeletal muscle. In experiment 339, for instance, even after long-continued search, not a single red blood cell was found. It is hardly necessary to state that normal unperfused skeletal muscle shows considerable numbers of red blood cells. It would seem that the skeletal muscles were particularly easily freed of blood by perfusion. It must be that the conditions of circulation here are such that a free flow of saline to all of the finer ramifications of the blood vessels is quite readily accomplished. Curiously enough the kidney with its double capillary circulation is also left almost completely devoid of blood by perfusion. In contrast, other organs such as the gastro-intestinal tract can be thoroughly perfused only with difficulty. Still in most cases we have been able to remove the blood almost entirely. The spleen and bone marrow are unique in that they possess blood cells which are not in direct communication with the blood stream. A review of the experiments presented shows that red blood cells are made out in fairly large numbers in these tissues.

TABLE 17

Experiment 351. Dog; 9.00 kgm. Hematocrit 41.1 per cent red blood cells

	TOTAL HEMOGLOBIN	TOTAL R. B. C.	BLOOD	PLASMA	PER 100 GRAMS BODY WEIGHT			
					Hemoglobin	R. B. C.	Blood	Plasma
	grams	*cc.*	*cc.*	*cc.*	*grams*	*cc.*	*cc*	*cc.*
Dye method	119.0	381.0	928.0	**538.0**	1.32	4.23	10.31	**5.98**
Carbon monoxide method	**111.0**	**358.0**	871.0	505.0	**1.23**	**3.98**	9.68	5.61
Welcker method								
Total bleedings	**95.0**	**306.0**	744.0	432.0	**1.06**	**3.40**	8.27	4.80
Extracts								
Heart	**0.14**	**0.45**	1.10	0.64	**0.0016**	**0.0050**	0.0122	0.0071
Spleen	**0.24**	**0.76**	1.86	1.08	**0.0027**	**0.0084**	0.021	0.012
Liver	**0.61**	**1.97**	4.8	2.8	**0.0068**	**0.022**	0.053	0.031
Skeletal muscles	**8.3**	**27.0**	65.0	38.0	**0.092**	**0.30**	0.72	0.42
Total extracts	**9.3**	**30.0**	73.0	43.0	**0.103**	**0.33**	0.81	0.47
Total (including extracts)	**104.0**	**336.0**	817.0	475.0	**1.16**	**3.73**	9.08	5.27

Lungs, liver, kidneys and gastro-intestinal tissue contain only traces of hemoglobin.

Experiment 351. Dog 20-72. Short-haired female mongrel bull terrier. In good condition. Weight, 9.00 kgm. Blood volume determination by dye method at 8:30 a.m.; by the carbon monoxide method at 8:40 a.m. Bleeding and perfusion under ether anesthesia in the usual manner begun at 9:30 a.m. Total duration of perfusion was 2 hours. A total of 18,010 cc. of blood-containing fluid was recovered. The hemoglobin estimations were made in the usual manner.

At autopsy the myocardium is pale. The lungs are quite well perfused except for several small irregular red patches. The spleen is quite small but otherwise appears normal. In areas scattered irregularly over the surface of the liver blood can be seen at the centers of the lobules. On section the liver is pale grey and cloudy. The lobulation is quite indistinct. The stomach shows but the faintest traces of macroscopic blood. The mucosa of the entire small intestine is of a brick-red color. An excess of mucus is present. The large intestine appears to be bloodless. The pancreas is purplish grey and quite edematous. The kidneys and pelvic organs are free of macroscopic blood.

In microscopic sections no red blood cells are seen in the section of myocardium. Sections of lung show a few red blood cells. Blood cells are very numerous in the spleen. The liver is cloudy. Its lobulation is poor. No red blood cells are seen. The villi of the small intestine are quite rich in red blood cells, although but few are to be seen deeper in the mucosa. No signs of blood seen in sections of the large intestine, kidney or skeletal muscle. A moderate number of red blood cells is seen in the pancreas.

The organs and tissues were extracted in the usual manner (see table 17).

In table 18 the quantity of hemoglobin extracted from the various organs is charted. The averages agree fairly well with the findings in the experiments already presented. The amount of hemoglobin is expressed in terms of body weight. It was impossible in this series to express the hemoglobin from each organ in terms of the total weight of that organ on account of the fact that most of the organs were quite edematous from perfusion and it was impossible to gain any very accurate notion of the correct weights of these organs. However, we have noted the weights of the various organs from other dogs killed for other reasons. Assuming that the same figures hold for this series of dogs we may calculate from table 18 that the lungs (normal weight 115 grams) must have contained about 0.01 gram of hemoglobin per 100 grams of tissue. The gastro-intestinal tract (normal weight about 500 grams) being as a rule somewhat less perfectly perfused, contained 0.015 gram per 100 grams of tissue. The liver (average weight about 420 grams) contains about 0.013 gram per 100 grams of liver substance. The spleen which is so rich in residual blood contains about 0.10 gram per 100 grams of tissue or approximately 0.25 gram for the entire organ. This same table shows that the organ extracts have a total of 0.126 gram of hemoglobin per 100 grams of body weight. Of this 0.103 gram or 82 per cent of the total is derived from the skeletal muscles. This seems rather high when it is recalled that microscopic study showed the muscles to contain so few red blood cells. Furthermore, using the results of table 18 as a basis it may be computed that from each 100 grams of skeletal muscle 0.02 gram of hemoglobin was extracted. This is little more than was recovered from a like quantity of lung tissue, but much less than was recovered from the same amount of splenic tissue. Still when it is recalled that the skeletal muscles constitute nearly one-half of the total body weight it will be seen that the total amount of pigment so recovered must of necessity constitute a large part of the total extracts. The relatively large amount of hemoglobin removed from the skeletal muscles by extraction must be derived only to a small extent from blood contained in this tissue. There can be little doubt that the greater part of this pigment is not blood hemoglobin but muscle hemoglobin. The existence within the striped muscle of such a pigment is well known. It has been shown that it is very similar to, if not identical with, blood hemoglobin (11).

From the bones 0.020 gram of hemoglobin per 100 grams of body weight was recovered. Much of this likewise cannot be considered as belonging to the circulating blood. Part of this exists in extravascular

TABLE 18

Summary of hemoglobin values in Welcker experiments

EXPERIMENT	WEIGHT OF DOG	HEMATOCRIT (R. B. C.)	HEMOGLOBIN PER 100 GRAMS OF BODY WEIGHT RECOVERED FROM									
			Total bleedings plus perfusions	Organ extracts								Total bleedings plus perfusions and extracts
				Heart	Lungs	Spleen	Liver	Stomach and intestines	Skeletal muscles	Bones	Total extracts	
	kgm.	*per cent*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
330	4.75	45.3	1.26			0.0044	0.0063	0.0103	0.101	0.033	0.160	1.42
356	8.77	39.8	0.88	0.0024	0.0013	0.0027			0.11	0.025	0.14	1.02
335	8.86	46.2	0.77	0.0015		0.0008	0.0031		0.087	0.005	0.097	0.87
351	9.00	41.1	1.06	0.0016		0.0027	0.0068		0.092		0.103	1.16
333	9.18	53.3	1.24	0.0031		0.0039			0.099	0.027	0.133	1.37
340	10.45	52.1	0.93	0.0015		0.0016			0.146	0.015	0.164	1.09
328	10.46	48.8	1.19		0.0011	0.0021			0.104		0.107	1.30
346	10.68	62.0	1.17	0.0010		0.0014			0.070	0.021	0.093	1.26
342	11.82	48.3	1.18	0.0015		0.0015			0.113	0.015	0.131	1.31
344	13.64	58.9	1.30	0.0014	0.0007	0.0018			0.075	0.018	0.097	1.40
345	13.64	59.5	1.34	0.0013		0.0012			0.094	0.018	0.114	1.45
339	15.45	50.1	1.19	0.0015		0.0017			0.147	0.021	0.171	1.36
349	16.36	46.4	1.17	0.0015		0.0008			0.097	0.029	0.128	1.30
343	18.64	58.0	1.39	0.0006	0.0006	0.0021	0.0018	0.0016	0.102	0.012	0.121	1.51
Average..	11.55	50.7	1.14	0.0016	0.0009	0.0021	0.0045	0.0060	0.103	0.020	0.126	1.27

bone marrow cells which have not yet matured and been thrown into circulation. The spleen contains many red blood cells which are not in active circulation. These cannot be removed by any amount of perfusion. The liver is of a brownish tint due to pigments peculiar to the liver. The presence of these pigments makes it difficult to estimate colorimetrically its blood hemoglobin content unless the latter be fairly large.

In many experiments some of the organs were so well perfused that by extraction such small amounts of hemoglobin were recovered that their quantitative estimation was impossible. In these cases no figures at all are entered in the tables. For this reason the figure for total extracts is slightly smaller than it would be if it had been possible to include the extracts of all organs.

In view of all of these facts obviously considerable difficulty surrounds an attempt to state with any degree of certainty just how much of the total hemoglobin extracted should be considered to be derived from blood cells left behind in the vessels after completion of perfusion. It would seem that the blood hemoglobin must constitute somewhat less than one-half of the total pigment extracted. If, however, we add a small amount for incomplete extraction of the tissues we will probably not err greatly in assuming such a figure. One-half of the total extracts would be 0.063 gram of hemoglobin per 100 grams of body weight. If this amount of hemoglobin be added to that obtained by bleeding and perfusion combined (1.14 grams per 100 grams of body weight) we have a total of 1.20 grams of *blood hemoglobin* per 100 grams of body weight. Even though our assumption that one-half of the pigment extracted is blood hemoglobin is somewhat erroneous, still this figure of 1.20 will be but slightly affected, for a difference of only 5 per cent results, if we neglect the extracts altogether or if we assume the other extreme—viz., that *all* of the extracted pigment is blood hemoglobin.

Muscle hemoglobin and other extravascular pigments of similar nature furnish subject for debate not only in the case of the Welcker method but in the carbon monoxide method as well. The muscle hemoglobin has been shown (11) to be very similar to that of blood hemoglobin and on being placed in contact with carbon monoxide it unites with the latter to form a substance having a spectrum similar to that of the carbon monoxide hemoglobin derived from blood hemoglobin. It seems quite probable that the carbon monoxide, which is administered in the carbon monoxide method and is taken up by the red blood cells, must through processes of diffusion reach and combine to some extent

TABLE 19

Summary of blood volume and hemoglobin values

EXPERIMENT	WEIGHT OF DOG	HEMATOCRIT (R.B.C.)	PER 100 GRAMS BODY WEIGHT														
			Hemoglobin				Red blood cells				Whole blood				Plasma		
			Dye method	CO method	Welcker method		Dye method	CO method	Welcker method		Dye method	CO method	Welcker method		Dye method	CO method	Welcker method
					With extracts	Without extracts			With extracts	Without extracts			With extracts	Without extracts			With extracts
	kgm.	*per cent*	*grams*	*grams*	*grams*	*grams*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
330	4.75	45.3	2.11	**1.96**	**1.42**	**1.26**	5.68	**5.28**	**3.83**	**3.41**	12.53	11.68	8.48	7.52	**6.74**	6.29	4.55
356	8.77	39.8	1.14	**1.03**	**1.02**	**0.88**	3.64	**3.30**	**3.22**	**2.78**	9.14	8.27	8.12	7.00	**5.30**	4.80	4.70
335	8.86	46.2	1.24	**0.76**	**0.87**	**0.77**	4.66	**2.84**	**3.26**	**2.89**	10.08	6.15	7.03	6.24	**5.27**	3.22	3.67
351	9.00	41.1	1.32	**1.23**	**1.16**	**1.06**	4.23	**3.98**	**3.73**	**3.40**	10.31	9.68	9.08	8.27	**5.98**	5.61	5.27
333	9.18	53.3	1.42	**1.67**	**1.37**	**1.24**	4.79	**5.65**	**4.65**	**4.19**	8.99	10.60	8.71	7.86	**4.10**	4.84	3.97
340	10.45	52.1	1.51	**1.13**	**1.09**	**0.93**	5.08	**3.79**	**3.67**	**3.13**	9.75	7.27	7.06	6.01	**4.58**	3.41	3.32
328	10.46	48.8	1.52	**1.20**	**1.30**	**1.19**	5.15	**4.07**	**4.38**	**4.02**	10.55	8.34	8.97	8.22	**5.28**	4.17	4.48
346	10.68	62.0	1.58	**1.42**	**1.26**	**1.17**	6.16	**5.53**	**4.90**	**4.54**	9.94	8.93	7.90	7.32	**3.70**	3.32	2.93
342	11.82	48.3	1.79	**1.36**	**1.31**	**1.18**	5.58	**4.25**	**4.09**	**3.67**	11.57	8.79	8.45	7.60	**5.76**	4.37	4.20
344	13.64	58.9	1.97	**1.44**	**1.40**	**1.30**	6.59	**4.82**	**4.69**	**4.36**	11.19	8.18	7.96	7.41	**4.51**	3.30	3.20
345	13.64	59.5	1.69	**1.35**	**1.45**	**1.34**	5.78	**4.62**	**4.98**	**4.59**	9.72	7.76	8.34	7.68	**3.85**	3.07	3.31
339	15.45	50.1	1.79	**1.44**	**1.36**	**1.19**	5.55	**4.47**	**4.22**	**3.68**	11.07	8.92	8.40	7.33	**5.42**	4.37	4.11
349	16.36	46.4	1.33	**1.28**	**1.30**	**1.17**	5.02	**4.46**	**4.51**	**4.06**	10.83	9.60	9.71	8.74	**5.69**	5.05	5.12
343	18.64	58.0	2.08	**1.51**	**1.51**	**1.39**	6.04	**4.37**	**4.41**	**4.05**	10.42	7.53	7.60	6.98	**4.29**	3.10	3.13
Average	11.55	50.7	1.61	**1.34**	**1.27**	**1.15**	5.28	**4.39**	**4.18**	**3.77**	10.39	8.69	8.27	7.44	**5.03**	4.21	4.00

with the muscle hemoglobin. It seems more than likely that the carbon monoxide method indicates not only the blood hemoglobin but takes in the muscle hemoglobin as well. We know of no other compound in the mammalian body which possesses an especial affinity for carbon monoxide. By physical solution at the carbon monoxide tension obtaining in the plasma of blood one-half saturated with carbon monoxide not more than 2 or 3 cc. of this gas could be taken up by all of the body fluids, even assuming the latter to constitute a very great proportion of the body. The error arising from this source would not exceed 2 or 3 per cent. It is improbable that this amount of CO could be held in body fluids in the presence of unsaturated hemoglobin.

Further study of table 19 shows in general the same figures for hemoglobin and red cells by the CO method and the Welcker method. The average figures show that the CO method gives figures which are 5 per cent greater than those recorded by the Welcker method for hemoglobin and tissue extracts combined. In general the figures correspond within reasonable limits. It is more simple to explain the occasional higher Welcker figures because we can assume incomplete equilibrium and mixing of the circulating CO. When the CO figures are higher than the Welcker figures we must assume some limitations in the accuracy of the technical procedure. The first experiment in table 19 (no. 330) is the only one which shows a startling discrepancy between the CO and Welcker figures. We have observed in other experiments that small or young dogs apparently had an unusual capacity of removal of CO from the blood stream. This point is being investigated further. Other than this the probability of a technical error must be considered as the figure of 5.28 cc. per 100 grams body weight is much above the average of 4.39. We see, however, that individual variations in this group of dogs is quite considerable—5.65 cc. per 100 grams, a maximum and 2.84 cc. per 100 grams, a minimum—average of 4.39 cc. This shows 100 per cent fluctuation between minimum and maximum. We believe that the accumulation of more data will show that the larger dogs show less variation in blood volume factors. These variations are not associated with anemia as shown by the hematocrit column. The plasma volume figures show less notable fluctuations—a maximum of 6.74 cc. per 100 grams body weight—a minimum of 3.70 cc. and an average of 5.03 cc.

From table 19 it is seen that 1.34 grams of hemoglobin per 100 grams of body weight are found by the carbon monoxide method. This is considerably higher than the amount which could be recovered in the

Welcker method by bleeding and perfusion combined. If, however, the total extracts are included in the Welcker determination we have a figure which approximates the value of 1.34 obtained by the carbon monoxide method. If experimental errors be taken into account these two values may be considered as almost identical. That this total hemoglobin (blood plus muscle hemoglobin) of the Welcker method should approximate the total hemoglobin, as determined by the carbon monoxide method, is evidence in favor of the view just outlined that by the carbon monoxide method the muscle hemoglobin as well as the blood hemoglobin is estimated.

DISCUSSION

Before attempting further to compare and correlate these three blood volume methods let us emphasize what has already been stated, viz., that the values *directly* obtained by each of the methods are in all cases tabulated in bold type. These figures include for the dye method the plasma volume; for the carbon monoxide and Welcker methods, the figures for hemoglobin and red blood cell volume. All other figures are derived *indirectly* from these figures on the *assumption* that the red blood cell volume bears a ratio to the plasma volume identical to the ratio of reds to plasma observed in a centrifugalized sample of blood drawn from the jugular vein. In this assumption is involved the idea that the plasma and red blood cells are thoroughly and uniformly mixed within the vascular system and that a sample of blood taken from the jugular vein contains neither more nor less than its proper proportion of red blood cells. But this assumption falls to the ground if it can be shown that conditions in certain parts of the vascular system (e. g., certain of the smaller vessels) are such as to favor undue retention of either plasma or red blood cells. We shall discuss presently certain evidence in favor of the view that the blood is far from being uniformly mixed. At any rate while the assumption mentioned above is under suspicion it is well to trust only the figures placed in bold type. Most previous investigators have unreservedly accepted the other figures also. These figures are included in our charts so that our results may be conveniently compared with and correlated with the results of previous workers.

We believe that the evidence already presented entitles one to conclude that the carbon monoxide method or the Welcker method furnished fairly reliable data concerning the total red blood cell volume. As has been noted above the maximum figures by either method in all

probability represent a figure which is somewhat greater than the true red blood cell volume. For in the Welcker method a part of the pigment extracted is not blood hemoglobin, but muscle hemoglobin. This muscle hemoglobin probably unites with carbon monoxide and thereby causes the carbon monoxide method to give values that are equally too high. If we deduct from the results of both methods an amount equal to one-half of the total organ extracts we shall have a red cell volume of 4.18 cc. per 100 grams of body weight by the carbon monoxide method and 3.97 by the Welcker method (see table 19). At any rate in round numbers 4.1 cc. per 100 grams must represent a very close approximation to the true red blood cell volume.

It must be emphasized repeatedly that the volume of *actively circulating red cells* is definitely less than the total *body hemoglobin* which, of course, includes muscle hemoglobin (Myohematin) and red cells in stagnant sinusoids in spleen and red marrow. It appears probable that this *immobile hemoglobin* represents 5 to 10 per cent of the total body hemoglobin which is concerned in the dilution of inhaled carbon monoxide. The CO method does not permit us to differentiate between the rapidly circulating hemoglobin and the immobile or fixed hemoglobin. Perhaps some method may be devised by which this difficulty can be surmounted.

The proof as to the size of the *plasma volume* is somewhat more difficult to obtain. To summarize, there are many methods for the determination of plasma volume. In all of them the principle is the same as the principle of the dye method. Dyes other than vital red have been used. Acacia, gelatin, dextrose, antitoxin and hemoglobin may also be substituted for the dye. All of these methods agree in indicating that the plasma volume is of the magnitude stated (4.8 cc. per 100 grams of body weight). It is difficult to believe that all of the substances thus used for the determination of plasma volume would be eliminated to any great extent in the 4-minute period allowed for their admixture with the plasma. Especially difficult is it to believe that substances so diverse in nature would all be so much more rapidly eliminated in the first few seconds than thereafter. The fact that dye once introduced can be recovered in large measure by perfusion indicates that destruction or elimination of dye is not great. Furthermore, the elimination of dye from circulation may be studied by withdrawing samples of blood for analysis at varying intervals of time following injection of the dye. A curve may be plotted to indicate these values. If this curve be drawn backward to cover the period prior to the taking of the 4-minute sample the result indicates that not more than 5 per cent

of the total dye injected is eliminated within the first 4 minutes. If a correction be made for this, the method shows that there are 4.8 cc. of plasma per 100 grams of body weight. We hope very soon to present experimental data to supplement this brief review of the plasma volume factors.

By *blood volume* is meant the sum *total of plasma volume, total red blood cell volume* and *white blood cell volume.* We have just discussed a method which determines plasma volume (dye method) and two others (carbon monoxide, Welcker) which determine red blood cell volume. No thoroughly reliable data concerning the total volume of white blood cells exists. Our hematocrit figures indicate that on an average the white blood cells constitute about 1.3 per cent by volume of the circulating blood. The work of Brodin, Richet and Saint-Girons (12) indicates that only about 50 per cent of all the white blood cells are in active circulation, the others being retained presumably in some of the smaller vessels. On the basis of these observations the white blood cell volume must constitute about 2.3 per cent of the total blood. If so, there are approximately 0.21 cc. of white blood cells per 100 grams of body weight. In this case the blood volume would equal per 100 grams of body weight, 4.8 cc. of plasma plus 4.2 cc. of red blood cells plus 0.2 cc. of white blood cells—a total of 9.2 cc. This we feel represents fairly accurately the total blood volume in our series of dogs. We wish to emphaszie our belief that it is only by a combination of methods that a proper estimate of the blood volume can be obtained. There is no justification for the custom of assuming that the plasma volume and red blood cell volume bear to each other the same ratio as is found in a centrifugalized sample of blood drawn from any of the usual sources. Yet this is exactly what is done when by the use of the hematocrit the total red blood cell volume is derived on the basis of a plasma volume previously determined by the dye method or the acacia method or any of the other plasma volume methods. It is equally incorrect to attempt to estimate the plasma volume when merely the red blood cell volume has been determined by either the carbon monoxide method or the method of Welcker. Such estimations involve the assumption that the ratio of red blood cells to plasma in blood drawn from the jugular vein is a ratio which is representative of the blood as a whole. A great amount of work has been reported which indicates that the distribution of blood cells in the body is a very changeable factor. The well-known observations of Cohnstein and Zuntz (13) may be cited in this regard. By microscopic study of living preparations they found that the smaller

vessels became crowded with red blood cells at the expense of plasma under certain conditions of vascular relaxation. Under conditions of vasoconstriction the red blood cells became less numerous and these fine vessels contained relatively more plasma. Much of the work on red blood cell count changes with high altitude and with exercise and the like may be explained on the basis of altered distribution of the blood cells. Confirmation of such theories is lacking, for as yet no investigator has studied these problems with a combination of a plasma volume method and a red blood cell method. The mere fact that while the red blood cell count rises, the red blood cell volume (or the total oxygen capacity of the blood) is unaltered does not indicate what changes may be occurring in the total volume of plasma. The work of Lamson shows that under certain conditions the blood cells may tend to accumulate in the liver (14).

We believe that the results of the present paper indicate in a way hitherto unsuspected, the variable admixture of the plasma and red blood cells. A review of table 19 shows that on an average there are 5.03 cc. of plasma per 100 grams of body weight. As has been already explained it seems probable that about 4 or 5 per cent of the dye injected is eliminated in the 4 minutes allowed for mixing of the dye with the plasma. Granting that this is true the corrected plasma volume is about 4.8 cc. of plasma per 100 grams of body weight. According to the same table there are about 4.2 cc. of red blood cells per 100 grams of body weight. The plasma together with the red blood cells equals the sum of these two figures or 9.0 cc. per 100 grams of body weight. If to this the probable white blood cell volume (0.2 cc. per 100 grams of body weight) be added, a sum-total of 9.2 cc. per 100 grams of body weight is obtained. This represents, we believe, a close approximation of the total blood volume. Since the red blood cells constitute 4.2 cc. of this 9.2 cc. it is easily seen that the red blood cells constitute 45.5 per cent of the total blood. This value represents the true red blood cell hematocrit. If, for instance, all of the plasma and blood cells could be removed from the body, placed in a large container and completely mixed, a sample of this blood on centrifugalization would be found to contain 45.5 per cent of red blood cells. In the living animal it is impossible to secure any such ideal sample, since it is impossible to take blood from all parts of the vascular system at once. A sample of blood taken from the jugular vein contains 50.7 per cent of red blood cells (table 19)—a figure considerably higher than the true 45.5 per cent. It would seem then that blood taken from the jugular vein is consider-

ably too rich in red blood cells. In an effort to locate other regions having a corresponding poverty in red blood cells we have drawn samples from the femoral vein, carotid artery, femoral artery, portal vein and other large vessels. Needless to say obstruction to the flow during the taking of all such samples was carefully avoided. It is not necessary to present all of the figures in detail. It will suffice to say that blood drawn from all of these sources was found within very narrow limits to contain the same percentage of red blood cells as was found in blood drawn from the jugular vein. We next turned our attention to the smaller vessels. It is very difficult to obtain any very exact information regarding the richness in red blood cells of the blood contained in such vessels. However, certain microscopic findings enable us to draw some general conclusions. Almost as soon as the microscopic circulation of the blood was first discovered it was observed that the flow in the smaller vessels was not at a uniform rate in all parts of the cross section. It was noted that in the central or axial portion of the stream the flow is much more rapid than in the peripheral portion of the stream. Furthermore the red blood cells are to be seen almost exclusively in the central rapidly moving part of the stream. If the laws governing the flow of simple liquids in tubes hold here, the center of the stream moves at a rate equal to twice the average velocity of the stream. The "still space" represents a body of plasma which is relatively stagnant—withdrawn from the circulation, as it were. The width of this layer undoubtedly varies in different vessels and under different conditions of flow. It is ordinarily stated that this layer has a width of at least one-eighth of the diameter of the vessel. If this be so it can easily be shown that this "still space" constitutes about 44 per cent of the cross sectional area of the vessel. Of course the plasma in this "still space" is not perfectly stationary and hence is not completely withdrawn from circulation. The rate of movement in any part of the vessel is approximately inversely proportional to its distance from the center. At this rate the plasma in the inner fourth of this so-called "still space" may be thought of as moving at the same rate as the innermost or "axial core" while the outer three-fourths of the "still space (i. e., the three-fourths nearest the vessel wall) may then be considered as being perfectly stationary. If, then, the "still space" constitutes 44 per cent of the cross sectional area of the vessel and three-fourths of this "still space" can be considered as being stationary it follows that 33 per cent (75 per cent of 44 per cent) of the contents of the vessel is stagnant plasma. The other 67 per cent of the content

of these small vessels is the actively circulating blood of the body and in our series of animals contained 50.7 per cent by volume of red blood cells. On an average there were 4.1 cc. of red blood cells per 100 grams of body weight. If these cells were all actively circulating our hematocrit would indicate that they should be accompanied by 4.0 cc. per 100 grams of body weight of actively circulating plasma. Our experiments show that the sum total of plasma is about 4.8 cc. per 100 grams of body weight. The difference between 4.8 and 4.0 is 0.8—a figure which represents the number of cubic centimeters of stagnant plasma per 100 grams of body weight. If the true blood volume be 9.0 cc. per 100 grams of body weight it is seen that the stagnant plasma constitutes about 9 per cent by volume of the total blood. All of this plasma could be stored away in the "still spaces" of the smaller vessels on the assumption that such small vessels contained one-third of the total blood. For, as was estimated above, about 33 per cent of the content of the smaller vessels consists of stagnant plasma for which we must account. It does not seem unreasonable to believe that one-third of the total blood is contained in the small vessels possessing an axial stream, particularly when it is recalled that the total cross section of the finer arterioles and venules has been estimated to be several hundred times the cross section of the aorta. Furthermore, the length of some of the arterioles is considerable. The total combined content of all such vessels must be very great.

Unfortunately previous microscopic studies and measurements do not enable us to establish conclusively our theory that the total volume of the "still space" is great enough to account for all of the plasma which we have shown is stored somewhere. We hope within the near future to be able to collect definite first-hand data on this point. These considerations do, however, establish the fact that there is within the "still spaces" at least a considerable quantity of stagnant plasma.

We must not overlook the possibility that plasma may also be "stored" in other regions. Thus Cohnstein and Zuntz (13) held that there are capillaries which are filled with plasma to the exclusion of red blood cells. Some investigators may wish to maintain that a certain part of the extravascular lymph spaces are quite accessible to the dye and that the lymph constitutes some of the stagnant part of our "plasma volume." The evidence now at hand is somewhat against this view. Our theory involving the "still spaces" seems to cover all known facts both from a qualitative and quantitative standpoint. This view in no way conflicts with the work of those who wish to maintain that under certain

conditions of circulation, red blood cells may be stored or deposited to excess in certain parts of the vascular tree. Nor do we wish to deny that under certain conditions the plasma volume may vary to a considerable degree. Further evidence is being collected on these points.

We wish to emphasize the view already presented to explain why the total "blood volume" as obtained by the dye method constitutes 9.5 to 10.5 cc. per 100 grams of body weight, while the total "blood volume" obtained by the carbon monoxide or Welcker method constitutes but 8 cc. per 100 grams of body weight. For blood drawn from the jugular vein is composed of approximately equal parts of plasma and blood cells. Anyone who did not know of the plasma stored away in the smaller vessels might make the mistake of thinking that the blood volume was twice the plasma volume or twice the total blood cell volume. In case he had already established by the dye method the fact that there are 4.8 cc. of plasma per 100 grams of body weight, he would be led to conclude there there is twice this amount of blood i. e., 9.6 cc. of blood per 100 grams of body weight. On the other hand, if he had established by the carbon monoxide or Welcker method that the red blood cell volume is 4.1 cc. per 100 grams of body weight he would conclude that there are but 8.2 cc. of blood per 100 grams. As our discussion shows, such calculations are not based upon sound premises. These workers should combine the plasma volume obtained by the dye method with the red blood cell volume obtained by the carbon monoxide or Welcker method. The result would be 8.9 cc. per 100 grams of body weight. If desired, one might add 0.2 cc. for white blood cells. In this case the observer would conclude that there are 9.1 cc. of blood per 100 grams of body weight. This, we believe, represents a correct approximation of the actual blood volume.

SUMMARY

Three standard blood volume methods are compared by the same workers under uniform conditions with a carefully controlled technic. The dye method, the carbon monoxide method and a modified Welcker method are all used on the same dog within a short space of time. The comparative value of these figures is thereby enhanced.

The dye method determines with reasonable accuracy the plasma volume but not the red cell volume. The carbon monoxide or Welcker method determines the total hemoglobin or red cell volume but not the plasma volume.

The dye method in normal dogs gives a plasma volume of about 4.8 cc. of plasma per 100 grams body weight. The carbon monoxide method in normal dogs gives the red cell volume as 4.2 cc. per 100 grams body weight. There are probably about 0.2 cc. of white blood cells per 100 grams body weight. In our opinion the *correct blood volume* is the sum of these figures or 4.8 cc. plasma + 4.2 cc. red cells + 0.2 cc. white blood cells = *9.2 cc. blood volume* per 100 grams body weight.

We believe other investigators have fallen into error due to *calculation of the total blood volume* based on the red cell hematocrit—usually 50 per cent in normal dogs. This gives erroneous figures of about 10 cc. per 100 grams body weight for the dye method and about 8 cc. per 100 grams body weight for the carbon monoxide method.

This error comes from the assumption that the blood cells and plasma are uniformly mixed in all parts of the circulating system. There is sufficient evidence to point to an *excess of plasma* over red cells in arterioles and capillaries. The axial cell stream in arterioles, for example, gives a marginal "still space" in which a considerable amount of plasma may be stored.

The absolute value of circulating red cells must always be somewhat smaller than the carbon monoxide figures which express the total *body hemoglobin*, inclusive of muscle hemoglobin and hemoglobin in red marrow and spleen sinusoids. The amount of this *immobile body hemoglobin* is about 5 to 10 per cent of the total body hemoglobin.

The true *total blood volume* can be obtained by the use of the carbon monoxide method (red cell volume) plus the dye or similar method (plasma volume). The sum of these figures gives the true blood volume.

BIBLIOGRAPHY

(1) Hooper, Smith, Belt and Whipple: This Journal, 1920, li, 205.
(2) Whipple, Hooper and Robscheit: This Journal, 1920, liii, 151.
(3) Keith, Rowntree and Geraghty: Arch. Int. Med., 1915, xvi, 547.
(4) Dawson, Evans and Whipple: This Journal, 1920, li, 232.
(5) McQuarrie and Davis: This Journal, 1920, li, 257.
(6) Arnold, Carrier, Smith and Whipple: Paper V of this series, This Journal.
(7) Salvesen: Journ. Biol. Chem., 1919, xl, 109.
(8) Welcker: Vierteljahrschrift f. Praktische Heilkunde, 1854, xliv, 11.
(9) Larson and Bell: Journ. Inf. Dis., 1919, xxiv, 185.
(10) Palmer: Journ. Biol. Chem., 1918, xxxiii, 119.
(11) Kühne: Arch. f. pathol. Anat. u. Physiol., 1865, xxxiii, 79.
(12) Brodin, Richet et Saint-Girons: Journ. de Physiol. et de Pathol. Gén., 1919, xviii, 27.
(13) Cohnstein and Zuntz: Arch. f. d. gesammt. Physiol., 1888, xlii, 303.
(14) Lamson: Journ. Pharm. Exper. Therap., 1920, xvi, 125.

EXPERIMENTAL STUDIES ON THE REGULATION OF BODY TEMPERATURE

IV. The Maintenance of a Practically Uniform Temperature in Rabbits by the Elimination of Random Movements

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In a previous paper (1) I reported the normal temperature variations in rabbits and the temperature effects of operative procedures. The animals were kept in large boxes in the experimental room in order to avoid the disturbing effect of daily removal from the animal house. They were always handled so as to cause as little excitement as possible and were fed daily about 6 p.m., that is, the rabbits were kept uniformly undisturbed but were allowed freedom in moving at will about the case (2 × 3 feet square). They thus generally indulged in moderate exercise.

Curve *A* is a specimen of the daily temperature variations of two rabbits under the above conditions, showing ranges of 0.7°–1.4°C. in the course of the day. In some cases the range was much greater, one varying 2.6°C. in one day. "The figures and curves show that one temperature observation cannot be used as the norm for that rabbit. A change above or below this one reading cannot be considered to be experimentally produced unless it is great enough to fall beyond the range of normal variability. Some workers consider a steady rise or fall as experimental compared with the fluctuating normal. It is conceivable that the range would need to be determined for any given experimental environment" (1).

In the same paper temperature variations were also given for rabbits which, for experimental purposes, have been bound back uppermost to an operating board. Under these conditions a marked fall, 1–2°C. or more, was recorded, the temperature remaining constant after the low level had been reached. This constant level was, however, too low to be used as a basis for experimental changes.

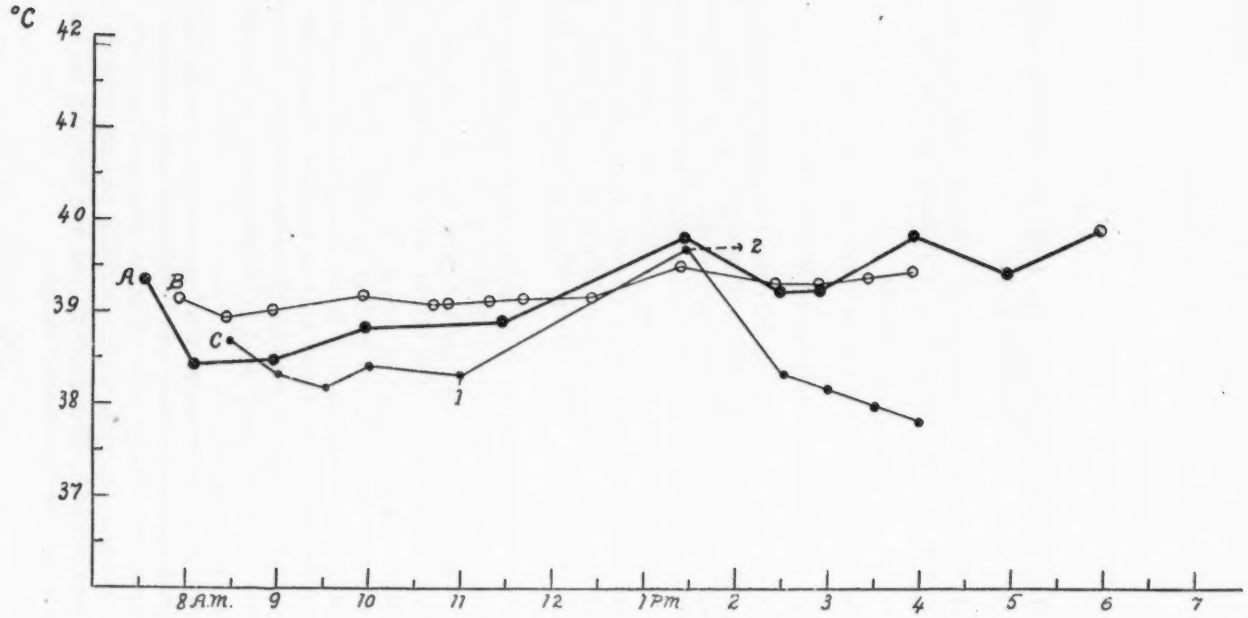

Fig. 1. *A*.—Normal temperature curve of rabbit in cage. *B*.—Normal temperature curve of rabbit in box. *C*.—Normal temperature curve of rabbit in box from beginning to *1*; loose in cage from *1–2*; in box from *2* to end.

The marked normal temperature variations indicated above tend to make the rabbit an unsatisfactory animal for the experimental studies of the regulation of body temperature. Further attempts were made, however, to establish an experimental environment which would result in a more constant temperature norm with less chance of fortuitous extremes. The result of this effort is the basis of the present paper.

Rabbits instead of being allowed freedom in a large cage were placed in a small box 6 × 18 inches long and 8 inches deep, in which they generally remained quiescent for hours. To insure absolute quiet, the head was placed in a Zwarmak head holder in one end of the box. In this position they appeared to be very contented and comfortable after the preliminary adjustments and would remain for hours without even an attempt at movement. There were exceptions, to be sure. Occasionally a male rabbit would make violent effort to escape. Those remaining quiet were assured of a less fluctuating temperature by the elimination of the effects of muscular exercise. Thus, one very pronounced variable which might easily be responsible for the wide range of normal variability was controlled, leaving only such as depend on food, sex, age, etc., all of minor importance. The pyretic effect of muscular exercise is a matter of every-day observation. Experimental exercise in rabbits has been reported by Krause (2) to cause a rise from 39.05°C. to 40.1°C. in 7 minutes. Thus chance movements, even though slight, would be expected to produce temperature changes of sufficient magnitude to mask many of experimental origin.

Curves of the temperature variations after the effects of muscular exercise have been eliminated show an almost constant temperature after the preliminary fall which marks the recovery from previous movement or that accompanying the process of adjustment in the box. The only apparent change in most cases was a gradual slight rise toward the end of the day. Marked fluctuations were never observed. In a number of cases the rabbits were removed from the box to a large cage after the temperature had been constant for some time. The release was invariably followed by an abrupt and marked rise as shown in curve *C*.

Curve *B* gives a specimen of the temperature variations of rabbits kept in the manner described above, in a box admitting of no muscular movement. The small range of variation and the lack of fluctuation are very evident. Thirty-one observations of daily variations were made on 19 rabbits with nearly identical results. A comparison of this curve with curve *A* of a rabbit at large in a cage shows in the former a nearly

uniform and non-fluctuating norm, departure from which could with a fair degree of certainty be ascribed to any known experimental cause; in the latter, large uncertain deviations of doubtful origin. It is true that many rabbits in the cages maintained a fairly constant temperature, as constant in fact as many in the boxes; but this condition was unpredictable. Those in the quiet of the box, though often showing some deviation, never in any instance had marked extremes.

I have used this method far more than two years on some 150 rabbits in my studies on the regulation of body temperature and have found that marked variations occur only after experimental procedure.

SUMMARY

Rabbits kept in small boxes with a Zwarmak head holder do not indulge in muscular exercise and therefore have a nearly constant body temperature. This makes it possible to study small experimental temperature changes with a degree of exactness not possible under the usual wide normal range of variability of rabbits not in a similarly controlled experimental environment.

BIBLIOGRAPHY

(1) MOORE: This Journal, 1918, xlvi, 244.
(2) KRAUSE: Arch. Exper. Path. u. Pharm. 1913, lxxii, 97.

EXPERIMENTAL STUDIES ON THE REGULATION OF BODY TEMPERATURE

V. The Temperature Effects of Different Concentrations of Sodium Chloride Solution Intravenously Administered

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I have shown in a former paper (1) that an increase in intracranial pressure is followed by a rise in body temperature in rabbits. The increase in pressure was brought about by mechanical means, but might be produced in various ways. The method of injecting salt solution intravenously to increase or decrease the cerebro-spinal pressure was suggested by the work of Weed and McKibben (2) in which it was shown that hypotonic solutions increase and hypertonic (saturated) solutions decrease the cerebro-spinal pressure in cats.

It seemed of interest to further test the possibility of a correlation between cerebro-spinal pressure and body temperature by observing the temperature effects of similar salt injections and their relation to the pressure effects noted by these workers. It was expected that the rise in the cerebro-spinal pressure produced in this manner would be paralleled by a rise in body temperature similar to that following mechanically increased pressure. The fall in cerebro-spinal pressure might correspondingly be accompanied by a fall in body temperature.

Five to 10 cc. of distilled water were used as the hypotonic solution and the same quantity or less of 6 m (saturated) NaCl as the hypertonic solution. Injections were made in the marginal vein of the ears of rabbits and caused no apparent discomfort or disturbance. Temperature readings were immediately begun and continued at 2 to 15 minute intervals until the maximum change had been reached and a return to normal had begun.

Since normal fluctuations are slight when the rabbit is fastened in the operating box (3) and as there were no variations due to anesthesia or operative procedures (4), a steady rise or fall in temperature followed

by a return to normal was considered as due to the effects of the salt solution injected.

Distilled water, curve *A*, caused a gradual average rise of 1°C. with a gradual return to normal. 6 m NaCl, curve *F*, which is practically a saturated solution, caused an abrupt average fall of 0.6°C. in 5 to 20 minutes followed by a rise to or above normal in 25 to 30 minutes. The marked initial fall in temperature is the characteristic effect of the 6 m NaCl. It occurred in 24 of the 30 cases of injection. The six rabbits in which a rise after injection of 6 m NaCl was observed instead of a fall had more or less severe convulsions following the administration. This is apt to happen if the injection is too rapid or the dose too large. The excessive muscular movements are no doubt responsible for the rise in temperature in these exceptional cases.

The increase in body temperature due to distilled water, hypotonic solution, and the fall due to 6 m NaCl, hypertonic solution, correspond roughly to the rise and fall in cerebro-spinal pressure following injection of the same concentrations as reported by Weed and McKibben (2).

Table of temperature changes following intravenous injections of NaCl

	NUMBER OF RABBITS USED	NUMBER OF INJECTIONS GIVEN	SUBSTANCE INJECTED	AMOUNT INJECTED	AVERAGE CHANGE IN TEMPERATURE	AVERAGE TIME PRECEDING MAXIMUM CHANGE
				cc.	*°C.*	*minutes*
1	2	3	Distilled water	5–10	+1.0	120
2	3	10	M/6 NaCl	5–10	+1.25	90
3	3	10	M/1 NaCl	5	+1.4	60
4	2	7	2 M NaCl	5	+1.5	60
5	3	8	4 M NaCl	5	+1.5	60
6	15	30	6 M NaCl	4–10	−0.6	20
					+0.4	90

In addition to the temperature effects of hypotonic and very concentrated hypertonic NaCl solutions, those of isotonic and more dilute hypertonic solutions were observed; m/6 NaCl, curve *B*, caused a rise similar to that of distilled water; m/1, 2 m and 4 m NaCl, curves *C*, *D*, *E*, although hypertonic, were followed by a rise which came on more rapidly and was of greater magnitude than that of distilled water or m/6 NaCl. There was an average rather abrupt rise of 1°C. with m/1, a more abrupt rise of 1.5°C. with 2 m and 4 m NaCl. In a few cases the rise after 4 m NaCl was preceded by a slight initial fall. It will be seen that the point of reversal between a temperature increasing and a

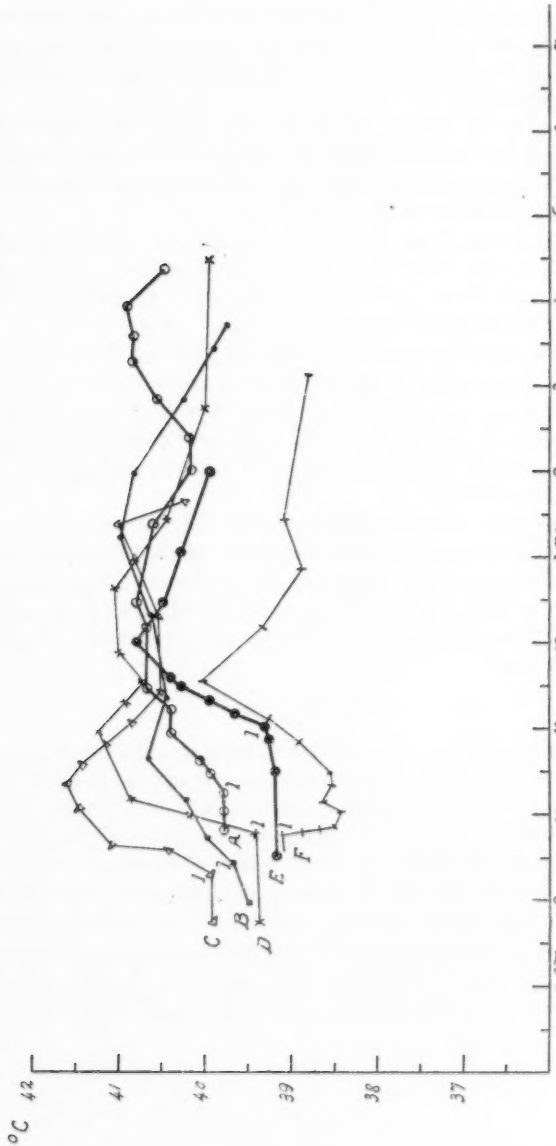

Fig. 1. *A.*—Temperature curve following injection of distilled water. *B.*—Temperature curve following injection of $\frac{M}{6}$ NaCl. *C.*—Temperature curve following injection of $\frac{M}{4}$ NaCl. *D.*—Temperature curve following injection of 2M NaCl. *E.*—Temperature curve following injection of 4M NaCl. *F.*—Temperature curve following injection of 6M NaCl.

temperature decreasing effect—the same quantity being used with each —is between 4 m and 6 m NaCl not between hypotonic and hypertonic solutions as would be expected from the effects of the same solutions on cerebro-spinal pressure.

The discussion of the results will be given in a later communication in connection with further data which are being obtained on the actual cerebro-spinal pressure, blood volume and vasomotor changes accompanying the rise and fall in body temperature.

It is of interest to note that while these experiments were in progress the work of Weed and McKibben (2) was repeated and their general results confirmed by Foley and Putnam (5), who also introduced the salt into the gastro-intestinal tract with similar results. The clinical application of the same principle was made by Cushing (6) who found that the brain volume could be altered by the administration of hypertonic salt solution per os and that the brain is peculiarly sensitive to movements of water in the tissues. Also Sach and Belcher (7) are using this method to decrease intracranial pressure before opening the dura in brain tumor operations.

Thus, it seems to be an established fact that the movement of water in the tissues due to changed osmotic conditions in the blood does alter the bulk of the brain and the cerebro-spinal pressure. And the evidence presented in this paper would seem to indicate that there is also a correlation between changes in the cerebro-spinal pressure and changes in body temperature.

SUMMARY

Distilled water, m/6, m/1, 2 m and 4 m sodium chloride in 5 to 10 cc. doses intravenously administered cause an average rise in body temperature of 1 to 1.5°C.; 6 m sodium chloride, on the other hand, causes an initial fall of 0.6°C. followed by a rise to or slightly above normal. The rise in temperature following the injection of hypotonic solutions corresponds roughly to the rise in cerebro-spinal pressure; and the fall following 6 m (hypertonic) sodium chloride to the fall in cerebro-spinal pressure after injections of hypotonic and hypertonic solutions as reported by Weed and McKibben. This seems to further indicate a correlation between cerebro-spinal pressure and body temperature.

BIBLIOGRAPHY

(1) Moore: This Journal, 1919, l, 102.
(2) Weed and McKibben: This Journal, 1919, xlviii, 512, 531.
(3) Moore: This Journal, 1921, lvi, 361.
(4) Moore, This Journal, 1918, xlvi, 244.
(5) Foley and Putnam: This Journal, 1920, liii, 464.
(6) Cushing: Prac. Soc. Exper. Biol. and Med., 1920, xvii, 217.
(7) Sachs and Belcher: Journ. Amer. Med. Assoc., 1920, lxxv, 667.